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(54) **THYROGLOBULIN QUANTITATION BY MASS SPECTROMETRY**

(56) **References Cited**

U.S. PATENT DOCUMENTS

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|--|--|-----------|-------------------|--------|----------------|-----------|---|--------|-----------------|-----------|----|--------|-------------------|-----------|----|--------|--------|--------------|----|--------|----------|--------------|----|--------|-----------------|--------------|----|---------|--------|--------------|----|--------|--------|--------------|----|--------|---------------|--------------|----|--------|------------------|
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Nigel J. Clarke, Oceanside, CA (US);
Richard E. Reitz, San Clemente, CA (US)</p> <p>(73) Assignee: Quest Diagnostics Investments Incorporated, Wilmington, DE (US)</p> <p>(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.</p> <p>This patent is subject to a terminal disclaimer.</p> | <table border="0"> <tr><td>6,107,623</td><td>A</td><td>8/2000</td><td>Bateman et al.</td></tr> <tr><td>6,124,137</td><td>A</td><td>9/2000</td><td>Hutchens et al.</td></tr> <tr><td>6,204,500</td><td>B1</td><td>3/2001</td><td>Whitehouse et al.</td></tr> <tr><td>6,268,144</td><td>B1</td><td>7/2001</td><td>Koster</td></tr> <tr><td>2004/0072251</td><td>A1</td><td>4/2004</td><td>Anderson</td></tr> <tr><td>2005/0064422</td><td>A1</td><td>3/2005</td><td>Barnidge et al.</td></tr> <tr><td>2006/0223188</td><td>A1</td><td>10/2006</td><td>Soldin</td></tr> <tr><td>2007/0105179</td><td>A1</td><td>5/2007</td><td>Madson</td></tr> <tr><td>2007/0224628</td><td>A1</td><td>9/2007</td><td>Gordon et al.</td></tr> <tr><td>2009/0042213</td><td>A1</td><td>2/2009</td><td>Hoofnagle et al.</td></tr> </table> | 6,107,623 | A | 8/2000 | Bateman et al. | 6,124,137 | A | 9/2000 | Hutchens et al. | 6,204,500 | B1 | 3/2001 | Whitehouse et al. | 6,268,144 | B1 | 7/2001 | Koster | 2004/0072251 | A1 | 4/2004 | Anderson | 2005/0064422 | A1 | 3/2005 | Barnidge et al. | 2006/0223188 | A1 | 10/2006 | Soldin | 2007/0105179 | A1 | 5/2007 | Madson | 2007/0224628 | A1 | 9/2007 | Gordon et al. | 2009/0042213 | A1 | 2/2009 | Hoofnagle et al. |
| 6,107,623 | A | 8/2000 | Bateman et al. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6,124,137 | A | 9/2000 | Hutchens et al. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6,204,500 | B1 | 3/2001 | Whitehouse et al. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6,268,144 | B1 | 7/2001 | Koster | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2004/0072251 | A1 | 4/2004 | Anderson | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2005/0064422 | A1 | 3/2005 | Barnidge et al. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2006/0223188 | A1 | 10/2006 | Soldin | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2007/0105179 | A1 | 5/2007 | Madson | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2007/0224628 | A1 | 9/2007 | Gordon et al. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2009/0042213 | A1 | 2/2009 | Hoofnagle et al. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

OTHER PUBLICATIONS

Communication pursuant to Article 94(3) EPC dated Jun. 22, 2012 issued in European Patent Application No. 08 860 014.3.

Bartolucci, et al., Liquid chromatography tandem mass spectrometric quantitation of sulfamethazine and its metabolites: direct analysis of swine urine by triple quadrupole and by ion trap mass spectrometry, *Rapid Commun. Mass Spectrom*, 14:967-73 (2000).

Bourrell et al., Immunoradiometric Assay of Thyroglobulin in Patients with Differentiated Thyroid Carcinomas: Need for Thyroglobulin Recovery Tests, *Clin. Chem. Lab Med*, 36(8):725-730 (1998).

(Continued)

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Related U.S. Application Data

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C12Q 1/37 (2006.01)
C12Q 1/00 (2006.01)

(52) **U.S. Cl.**
USPC **436/86; 436/171; 436/173; 435/6.19; 435/7.1; 435/23**

(58) **Field of Classification Search**
USPC **436/86, 171, 173; 435/7, 7.1, 6.19, 435/23**

See application file for complete search history.

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(57) **ABSTRACT**

Provided are methods for determining the amount of thyroglobulin in a sample using various purification steps followed by mass spectrometry. The methods generally involve purifying thyroglobulin in a test sample, digesting thyroglobulin to form peptide T129, purifying peptide T129, ionizing peptide T129, detecting the amount of peptide T129 ion generated, and relating the amount of peptide T129 ion to the amount of thyroglobulin originally present in the sample.

28 Claims, 15 Drawing Sheets

P01266 Sequence

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MALVLEIFTLASTGWVSANTFRYQVDAQPLRFCELORETFAPLKOADVVPQCAEDGSPOT
VQONDGSCWCWANGSEVLGSRQGRFVACLSPQIQKQQLLGGVINSTDTSYLPQC
QDGGDYAPVQCDVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQV
PCCSAGEFPMQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQVQV
QGRLEARTGLLELLDEIYDTIFAGLLDPSFTFTTLYRLLQRRFLAVQSVISGRFRCTPK
CEVERFTATSFGHPYVPCRRNGDYQAVQCOTBSPCWCVDQAGKEMHGTROQGEPPSCAE
GQSCASERQALSRYPGTSGYFSQHDLPSSPKRWASPRVARFATSCPTIKELFVDSG
LLRPMVBGQSQQFVSENLLKEAIRALFPRGLARLALQFTINPKRLQNLFGGKFLVNV
GQFNLSGALGTRGTFNFSQFFQQLGLASFLNGRQBEDLAKPLSVGLDSSNSTGTPEAAK
DGTMNKPTVGSFQFPEINLQENQALKFLASLLELPELPLFLQHALSVFEDVARDLGDVME
TVLSSQTCBQTPERLFPVPSCTTRGSDYEDVQCPSGECVNSWGLPGRVRRGGQPRCPT
DCEKQARMQSLMGSQVAGSTLFPVPACTSGHFLPVQCENSECYVDAEQALPQTRSAI
GPKKCPTECQLQSEQAFPLRTVQALLSNSMLPTLSDTYIPQCSTDQQRQVQVQVQVQV
VFELYQRWEAQNKQDQLTFAKLLVKIMSYREASGNFSLFIQSLYBAGQVQVFPVLSQYP
SLQVPLAALSGKRFPQPRENILLEPFLWQILNGLSQVYFSGYSDFSTFLAHFDLRNCWC
VDEAGQELBOMRSEPSKLPCTPGSCBEAKLRLVQPTRETTREIVSASNSRFFPLGSEFLVA
KGRIRLWEDLCLPLFPPEAFABQFLRSGDYAIRLANAQTLBFTYQRERFEDDSAGASA
LLRSGPYMQCDAFQSWHFPVQCHAGTCHKWCDEKGGFPGSLTARSLQTPQCTTCES
RTSGLLSKQARSQENSPKDLFVPACLTGTBYARIQASAGACTWCVDPASGELRFGSS
SSAQCSLNCVLSGVLRRVSPGVYVPAACAEDEGFSVQCDQAGQSCVMDSGSEVFG
TRVGGQPACEPRCLPFPNAGEVVGTTILCETISGPTGSAMOGCOLLCROGNSVFPFG
PLICSGESGRWESQLPQACRQRPQLWOTITQSHFQLQFPKMGCSADYADLITQVQV
ILDELTAGFCQTKVFTGLVSI PVCNNSVQVQVCLTRERLGVNVTWKRLEDITFVASL
PDLHDIERALVGDLLGRFTDLIQSGSFQLHLSKTFPAETIRFLQGDHFTSPTWFGC
SEGFPVQLTSEASQDGLGCVKCPGYSQDEBECPCPVGFYQEQAGSLACVCPVGRTTI
SAGAFSQTVCVTDQRNEAGLQCDQNGYRASQKDRGSGKAFVQVDSGRRLPWETRAPL
EDSQCLMMQKFEKVPESKVIQDANAPAVRASKVDESBFPVMQCLDCTEDRACGFPVST
TEPEISCDYAWTSNDVACMTSDQRDALGNSKATSPGSLRCOVKVRSHQDQSPAVYLLK
QGGSTTLQKRFEPTEFGQNLBGLYNIIVPSASGANLTAHLFCLLACDRDLCCDQFVLT
VQGGGAILICGLLSSPVLICNVKDNMDPSEAWANATCPGVTYDQESHQVILALGQSPFK
SLTFLBGTQDFTNFQVQVYLWQSDMGRPEMGCRCRKTVPFPASPTAAGLITTELPSFVD
LNGVTVNGQSLSSQKHWLPRHLFSAQANLWCLSKCVQEHSCQWAEITTEASLYFTCT
LYPEAQICDDIMSNWQCRLLPQMERALFKKVLLEDKVNFTLFPQRLAGSTFRN
KVPNSFKSISNGFBCBRCDADPCCTGFGFLNVSQKGGVYTCFLNLSLGLQKCSBNG
GAWRLDCGSDLEVHTYFPGVYQKPLIAGNAPSFCPLVVLBSLTKVSLDWSGLLSS
VYVDSIRHFDVAHVSTAATSNSPVAARDLCLSECSQHEACLETTLQTPGAVRCMFYADT
QSTHSLQGNCRLLREBATHYRKPGLSLLSEASVSPVISTHGRLLGRSQAIVGT
SWKVDQFLGVYAAPPLAHRFPQAPBPLNWTGSWDAKFRASCWQPGTSTSPGVSBD
CLYLVNFIQNVAPNASVLFPHNTMREBSEGPALDGSFLAAGVNLIVTASVYVGVF
GFLSBSGSEVSNWGLDQVAALTWQTHIRGFGDPRRVS LAADRGGADVASIHLLTAR
ATNSQLFRARVLMGGSALSAAVISHERAQQQAIALAKEVSPMSSSQEVVSCURQKPN
VLNDAQTKLLAVSGPFWYVIGHFLREPPARALKRSLWVVEVDLLIGSSQDDGLINRA
KAVKQFESRGRSTSKTAQYQALQNSLGEDSDARVBAATWYVSLSEHSTDDYASFBRAL
ENATRDYFIIICPIIDMASAWAKRARGNVFMYHAPENYHGSLLELADVQFALGLFPYBAY
EGQFSLKESLSLKIWQYPSHFTIRGNPNYPYBPRKVPPTFATPFDVFPFRAGGENYKEF
SELLPNRQGLKADCSFWSKYISLKTSDAGARKGQASBEBREHITAGSGLRELLSLQE
FGSKTYSK

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OTHER PUBLICATIONS

- Di Jeso, et al., Mixed-Disulfide Folding Intermediates between Thyroglobulin and Endoplasmic Reticulum Resident Oxidoreductases ERp57 and protein Disulfide Isomerase, *Molecular and Cellular Biology*, 25(22):9793-9805 (2005).
- Dunn et al., The Sites of Thyroid Hormone Formation in Rabbit Thyroglobulin, *The Journal of Biological Chemistry*, 262(35):16948-16952 (1987).
- Dunn et al., Tyrosine 130 is an Important Outer Ring Donor for Thyroxine Formation in Thyroglobulin, *J. Biol. Chem.*, 273(39):25223-25229 (1998).
- Gentile, et al, Identification of Hormonogenic Tyrosines in Fragment 1218-1591 of Bovine Thyroglobulin by Mass Spectrometry, *J Bio Chem*, 272(1):639-646 (1997).
- Hoofnagle, et al, Quantification of Thyroglobulin, a Low-Abundance Serum Protein, by Immunoaffinity Peptide Enrichment and Tandem Mass Spectrometry, *Clin Chem*, 54(11):1796-1804 (2008).
- International Preliminary Report on Patentability dated Jun. 8, 2010.
- International Search Report dated Apr. 22, 2009 for PCT Application No. PCT/US08/85435.
- International Communication dated Sep. 20, 2011 for EP Application No. EP 08860014.3.
- International Search Report Supplemental dated Jan. 28, 2011 for EPO Application No. 08860014.3.
- Kim et al., Folding and Assembly of Newly Synthesized Thyroglobulin Occurs in a Pre-Golgi Compartment, *The Journal of Biological Chemistry*, 266(19):12412-12418 (1991).
- Merchant, M. and S. R. Weinberger, Recent advancements in surface-enhanced laser desorption/ionization-time of flight-mass spectrometry, *Electrophoresis*, 21:1164-67, (2000).
- Persoon et al., Clinical Utility of an Automated Immunochemiluminometric Thyroglobulin Assay in Differentiated Thyroid Carcinoma, *Clinical Chem.*, 52(4):686-691 (2006).
- Persoon et al., Thyroglobulin (Tg) Recovery Testing with Quantitative Tg Antibody Measurement for Determining Interference in Serum Tg Assays in Differentiated Thyroid Carcinoma, *Clinical Chem.*, 52(6):1196-1199 (2006).
- Robb et al., Atmospheric pressure photoionization: An ionization method for liquid chromatography-mass spectrometry, *Anal. Chem.*, 72(15): 3653-3659.
- Salek et al., Analysis of thyroglobulin iodination by tandem mass spectrometry using ammonium ions of monoiodo- and diiodo-tyrosine, *Proteomics*, 5(2):351-353 (2005).
- Salm et al., The Quantification of Sirolimus by High-Performance Liquid Chromatography-Tandem Mass Spectrometry and Microparticle Enzyme Immunoassay in Renal Transplant Recipients, *Clin. Therapeutics*, 22 Suppl. B:B71-B85, (2000).
- Spencer et al., Detection of Residual and Recurrent Differentiated Thyroid Carcinoma by Serum Thyroglobulin Measurement, *Thyroid*, 9(5):435-41 (1999).
- Spencer et al., Thyroglobulin Measurement Techniques, Clinical Benefits, and Pitfalls, *Endocrinol Metab Clin North Am.*, 24(4):841-863 (1995).
- Taylor et al., Simultaneous Quantification of Tacrolimus and Sirolimus, in Human Blood, by High-Performance Liquid Chromatography-Tandem Mass Spectrometry, *Therapeutic Drug Monitoring*, 22:608-12, (2000).
- US Notice of Allowance dated May 11, 2011 for U.S. Appl. No. 12/001,076.
- US Office Action dated Apr. 28, 2010 for U.S. Appl. No. 12/001,076.
- US Office Action dated Oct. 27, 2010 for U.S. Appl. No. 12/001,076.
- US Office Action dated Jan. 25, 2011 for U.S. Appl. No. 12/001,076.
- Wright et al, Proteinchip surface enhanced laser desorption/ionization (SELDI) mass spectrometry: a novel protein biochip technology for detection of prostate cancer biomarkers in complex protein mixtures, *Prostate Cancer and Prostatic Diseases*, 2:264-76, (1999).
- Communication pursuant to Article 94 (3) EPC dated Feb. 13, 2012 received in EPO Application No. 08860014.3.

Figure 1

P01266 Sequence

MALVLEIFTLLASICWVSANIFEYQVDAQPLRPCELQRETAFLKQADYVPOCAEDGSFQT
VQCQNDGRSCWCVGANGSEVLGSRQPGRPVACL SFCQLQKQOILLSGYINSTDTSYLPQC
QDSGDYAPVQCDVQQVQCWCVDAEGMEVYGTRQLGRPKRCPRSCEIRNRLLHGVGDKSP
PQCSAEGEFMPVQCKFVNTTDMMI FDLVHSYNRFPDAFVTFSSFQRRFPEVSGYCHCADS
QGRELAETGLELLLDEIYDTIFAGLDLPSTFTETTLYRILQRRFLAVQSVISGRFRCPTK
CEVERFTATSFGHPYVPSRNRNGDYQAVQCQTEGPCWCVDAQGKEMHGTRQQGEPSCAE
GQSCASERQQALSRLYFGTSGYFSQHDLFSSPEKRWASPRVARFATSCPPTIKELFVDSG
LLRPMVEGQSQQFSVSENLLKEAIRAIFPSRGLARLALQFTTNPKRLOQNLFGGKFLVNV
GQFNLSGALGTRGTFNFSQFFQQLGLASFLNGGRQEDLAKPLSVGLDSNSSTGTPEAAKK
DGTMNKPTVGSFGFEINLQENQNALKFLASLLELPEFLFLQHAISVPEDVARDLGDVME
TVLSSQTCEQTPERLFPVPSCTTEGSYEDVQCFSGECWCVNSWGKELPGSRVRGGQPRCPT
DCEKQRARMQSLMGSQPAGSTLFPACTSEGHFLLPVQCFNSECYCVDAEGQAI PGTRSAI
GKPKKCPTPCQLQSEQAFLRTVQALLSNS SMLPTLSDTYI PQCSTDGQWRQVQCNGPPEQ
VFELYQRWEAQNKGDLTPAKLLVKIMSYREAASGNFSLFIQSLYEAGQODVFPVLSQYP
SLQDVPLAALEGKRPQPRENILLEPYLFWQILNGQLSQYPGSYSDFSTPLAHFDLRNCWC
VDEAGQELEGMRSEPSKLP TPCGSCEEAKLRVLQFIRETEEIVSASNSSRFPLGESFLVA
KGIRLRNEDLGLPPLFP PREAFAEQFLRGSDYAIRLAAQSTLSFYQRRRFS PDDSAGASA
LLRSGPYMPQCDAFGSWEVPVQCHAGTGHCWCVDEKGGFI PGSLTARSLQI PQCPTTCEKS
RTSGLLSSWKQARSQENPSPKDLFVPACLETGEYARLQASGAGTWCVDPASGEELRPGSS
SSAQCPSLCNVLKSGVLSRRVSPGYVPACRAEDGGFSPVQCDQAQGSWCVMDSGEEVPG
TRVTGGQPACESPRCPLPFNASEVVGTTILCETISGPTGSAMQQCQLLCRQGSWSVFP
PLICSLESGRWESQLPQPRACQRPQLWQTIQTQGHFQLQLPPGKMCSADYADLLQTFQVF
ILDELTARGFCQIQVKTFGTLVSI PVCNNS SVQVGLTRERLGVNVTWKSRLIEDIPVASL
PDLHDIERALVGKDLLGRFTDLIQSGSFQLHLDSKTFPAETIRFLQGDHFGTSPRTWFGC
SEGFYQVLTSEASQDGLGCVKCP EGSYSQDEECIPCPVGFYQEQAGSLACVPCPVGRTTI
SAGAFSQTHTCVDTCQRNEAGLQCDQNGQYRASQKDRGSGKAFCDVGEGRRLPWWETEAPL
EDSQCLMMQKFEKVPESKVI FDANAPVAVR SKVPDSEFPVMQCLTDCTEDEACSF FTVST
TEPEISCDFYAWTSDNVACMTSDQKRDALGNSKATSFGLRQCQVKVRSHGQDSPA VYLKK
GQGSTTTLQKRFEPTGFQNM LSGLYNPIVFSASGANLTD AHLFCLLACDRDLCCDGFVLT
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LNQVIVNGNQSLSSQKHWLFKHLFSAQQANLWCLSRCVQEH SFCQLAEITESASLYFTCT
LYPEAQVCD DIMESNAQGCRLILPQMPKALFRKKVILEDKVKNFYTRLPFQKLMGISIRN
KVP MSEKSI SNGFFECERRCDADPCCTGFGFLNVSQ LKGGVETCLTLNSLGIQMCSEENG
GAWRILDCGSPDIEVHTYPFGWYQKPIAQNNAP SFCPLVVLPSLTEKVS LDSWQSLALSS
VVVDPSIRHFDVAHVSTAATS NFSAVRDLCLSECSQHEACLITTLQTQPGAVRCMFYADT
QSCTHSLQGQNCRLLLREETHIYRKPGISLLSYEASVPSVPISTHG RLLGRSQAIQVGT
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CLYLN VFI PQNVAPNASVLVFFHNTMDRE ESEGWPAIDGSFLAAVGNLI VVTASYRVGVF
GFLSSGSGEVSGNWGLLDQVAALTWVQTHIRGFGG DRRVSLAADRGGADV ASIHL LTAR
ATNSQLFRRAVLMGGSALS PAAVISHERAQQQAIALAKEVSCPMSSSQEVV SCLRQK PAN
VLNDAQTKLLAVSGPFHYWGPVIDGHFLREPPARALKRSLWVEVDLLIGSSQDDGLINRA
KAVKQFEESRGR TSSKTAFYQALQNSLGGEDSDARVEAAATWYYSLEHSTDDYASFSRAL
ENATRDYFIICPIIDMASAWAKRARGNVFMYHAPENYGHGSLELLADVQFALGLPFYPAY
EGQFSLEEKSLSLKIMQYFSHFIRSGNPNYPYEF SRKVPTFATPWPDFVPRAGGENYKEF
SELLPNRQGLKKADCSFWSKYISSLKTSADGAKGGQSAESEEEELTAGSGLREDLLSLQE
PGSKTYSK

Figure 2

P01266-2 Isoform 2 Sequence

>sp_vs|P01266-2|THYG_HUMAN Isoform 2 of P01266 - Homo sapiens (Human)
MALVLEIFTLLASICWVSANIFEYQVDAQPLRCELQRETAFLKQADYVPQCAEDGSFQT
VQCQNDGRSCWCVGANGSEVLGSRQPGRPVACL SFCQLKQKQILLSGYINSTDTSYLPQC
QDSGDYAPVQCDVQQVQCWCVDAEGMEVYGTRQLGRPKRCPRSC EIRNRLLHGVDKSP
PQCSAEGEFMPVQCKFVNTTDMMIFDLVHSYNRFPDAFVTFSS FQRRFPEVSGYCHCADS
QGRELAETGLELLLDEIYDTIFAGLDLPSTFTETTLYRILQRRFLAVQSVISGRFRCPTK
CEVERFTATSFGHPYVPSRRNGDYQAVQCQTEGPCWCVDAQ GKEMHGTRQQGEPSCAE
GQSCASERQQALSRLYFGTSGYFSQHDLFSSPEKRWASPRVARFATSCPPTIKELFVDSG
LLRPMVEGQSQQFSVSENLLKEAIRAIFPSRGLARLALQFTTNPKRLQQNLFGGKFLVNV
GQFNLSGALGTRGTFNFSQFFQQLGLASFLNGGRQEDLAKPLSVGLDSNSSTGTPEAAKK
DGTMNKPTVGSFGFEINLQENQNALKFLASLLELPEFLFLQHAISVPEDVARDLGDVME
TVLSSQTCEQTPERLFPVPSCTTEGSYEDVQCFSGECWCVNSWGKELPGSRVRGGQPRCPT
DCEKQRRMQSLMGSQPAGSTLFPACTSEGHFLPVQCFNSECYCVD AEGQAI PGTRSAI
GKPKKCPTPCQLQSEQAFLRTVQALLSNSSMLPTLSDTYIIPQCSTDGQWRQVQCNGPPEQ
VFELYQRWEAQNKGD LTPAKLLVKIMSYREAASGNFSLFIQSLYEAGQQDVFPVLSQYP
SLQDVPLAALEGKRPQPRENILLEPYLFWQILNGQLSQYPGSYSD FSTPLAHFDLRNCWC
VDEAGQELEGMRSEPSKLP TPCPGSC EEA KLRVLQFIRETEEIVSASNSSRFPLGESFLVA
KGIRLRNEDLGLPPLFPREAF AEQFLRGSDYAIRLAAQSTLSFYQRRRFS PDDSAGASA
LLRSGPYMPQCDAFGSWEPVQCHAGTGHCWCVDEKGGFIPGSLTARSLOIPQCPTTCEKS
RTSGLLSSWKQARSQENPSPKDLFVPACLETGEYARLQASGAGTWCVD PASGEELRPGSS
SSAQCPSLCNVLKSGVLSRRVSPGYVPACRAEDGGFSPVQCDQAQ GSCWCVMDSGEEVPG
TRVTGGQPACESPRCPLPFNASEVVGTTILCETISGPTGSAMQOCQLL CRQGSWSVFPFG
PLICSLESGRWESQLPQPRACORPQLWQTIQTQGHFQLQLPPGKMCSADYADLLQTFQVF
ILDEL TARGFCQIQVKTFTGLVSI PVCNNS SVQVGC LTRERLGVNVTWKSRL EDIPVASL
PDLHDIERALVGKDLLGRFTDLIQSGSFQLHLD SKTFPAETIRFLQGDHFGTSPRTWFGC
SEGFYQVLTSEASQDGLGCVKCEPGSYSQDEECIPCPVGFYQEQA GSLACVPCPVGRTTI
SAGAFSQTHLMQKFEKVPESKVI FDANAPVA VRSKVPDSEFPVMQCLTDCTE DEACSF FT
VSTTEPEISCDFYAWTSDNVACMTSDQKRDALGNSKATSFGSLRCQVKVRSHGQDSPAVY
LKKGQGSTTTLQKRFEPTGFQNM LSGLYNP IVFSASGANLTD AHLFCLLACDRDLCCDGF
VLTQVQGGAIICGLLSSPSVLLCNVKD WMDPSEAWANATCPGV TYDQESHQVILRLGDQE
FIKSLTPLEGTQDTFTNFQQVYLWKDS DMGSRPESMGCRKDTVPRPAS PTEAGLTTELFS
PVDLNQVIVNGNQSLSSQKHWLFKHLFSAQQANLWCLSRCVQEHSFCQLAEITESASLYF
TCTLYPEAQV CDDIMESNAQGCR LILPQMPKALFRKKVILEDKVKNFYTRLPFQKLMGIS
IRNKVPMSEKSI SNGFFECERRCDADPCCTGFGFLNVSQ LKGG EVTCLTLNSLGIQMCSE
ENGGAWRILD CGSPDIEVHTYPFGWYQKPIAQNNAPSFCPLVVLPSL TEKVSLDSWQSLA
LSSVVVDPSIRHFDVAHVSTAATS NFSAVRDLCLSECSQHEACLITTLQTQPGAVRCMFY
ADTQSC THSLQGQNC RLLLREEATHIYRKPGISLLSYEASVPSVPISTHG RLLGRSQAIQ
VGT SWKQVDQFLGV PYAAPPLAERRFQAPEPLNWTGSWDASKPRASCWQP GTRTSTSPGV
SEDCLYLVNFI PQNVAPNASVLVFFHNTMDRE ESEGWPAIDGSFLAAVGNLIVVTASYRV
GVFGFLSSGSGEVSGNWGLLDQVAALTWVQTHIRGFGGDPRRVSLAADRGGADVASIHLL
TARATNSQLFRRAVLMGGSALS PAAVISHERAQQQAIALAKEVSCPMSSSQEVV SCLRQK
PANVLNDAQTKLLAVSGPFHYWGPVIDGHFLREPPARALKRSLWVEVDLLIGSSQDDGLI
NRAKAVKQFEESRGR TSSKTAFYQALQNSLGGEDSDARVEAAATWYYSLEHSTDDYASFS
RALENATRDYFIICPIIDMASAWAKRARGNVFMYHAPENYGHGSLELLADVQFALGLPFY
PAYEGQFSLEEKSLSLKIMQYFSHFIRSGNPNYPYEF SRKVPTFATPWPDFVPRAGGENY
KEFSELLPNRQGLKKADCSFWSKYISSLKTSADGAKGGQSAESEEEELTAGSGLREDLLS
LQEPGSKTYSK

Figure 3

Q59GF0 (Tg variant-Fragment) Sequence

>Q59GF0|Q59GF0_HUMAN Thyroglobulin variant (Fragment) - Homo sapiens (Human).
IPRKPISKRPVRPSLPRSPRCPLPFNASEVVGTTILCETISGPTGSAMQQCQLLCRQGSW
SVFPPGPLICSLESGRWESQLPQPRACQRPQLWQTTIQTQGHFQLQLPPGKMCSADYAGLL
QTFQVFILDELTAARGFCQIQVKTFGTLVSI PVCNNSVQVGCLTRERLGVNVTWKSRLD
IPVASLPDLHDIERALVGKDLLGRFTDLIQSGSFQLHLDSKTFPAETIRFLQGDHFGTSP
RTWFGCSEGFYQVLTSEASQDGLGCVKCEPGSYSQDEECIPCPVGFYQEQAGSLACVPCP
VGRTTISAGAFSQTCHCVTDCQRNEAGLQCDQNGQYRASQKDRGSGKAFVCDGEGRRLPWW
ETEAPLEDSQCLMMQKFEKVPESKVI FDANAPVAVRSKVPDSEFPVMQCLTDCTEDEACS
FFT VSTTEPEISCDFYAWTSDNVACMTSDQKRDALGNSKATSFGLRCQVKVRSHGQDSP
AVYLKKGQGSTTTTLQKRFEPTGFQNMLSGLYNPIVFSASGANLTD AHLFCLLACDRDLCC
DGFVLTQVQGGAIICGLLSSPSVLLCNVKDWM DPSEAWANATCPGVTYDQESHQVILRLG
DQEFIKSLTPLEGTQDTFTNFQOVYLWKDS DMGSRPESMGCRKNTVPRPASPT EAGLTTE
LFSVPDLNQVIVNGNQSLS SQKHWLFKHLFSAQQANLWCLSRVQEH SFCQLAEITESAS
LYFTCTLYPEAQVCDDIMESNAQGCRLLLPQMPKALFRKKVILEDKVKNFYTRLPFQKLT
GISIRNKVPMSEKSI SNGFFECERRCDADPCCTGFGFLNVSQ LKGGEVTCLTLNSLGIQM
CSEENGGAWRILD CGSPDIEVHTY PFGWYQKPIAQNNAPSFCPLVVLPSL TEKVSLDSWQ
SLALSSVVVDPSIRHFDVAHVSTAATS NFSAVRDLCLSECSQHEACLITTLQTQPGAVRC
MFYADTQSC'THSLQGQNC RLLLREEATHIYRKPGISLLSYEASVPSVPISTHGRLLGRSQ
AIQVGT SWKQVDQFLGVPYAAPPLAERRFQAPEPLNWTGSWDASKPRASCWQP GTRTSTS
PGVSEDCLYLNVFIPQNVAPNASVLVFFHNTMDREESEGWPAIDGSFLAAVGNLIVVTAS
YRVGVFGFLSSGSGEVSGNWGLLDQVAALTWVQTHIRGFGGDP RRVS LAADRGGADVASI
HLLTARATNSQLFRRAVLMGGSALS PAAVISHERAQQQAIALAKEVSCPMSSSQEVV SCL
RQKPANVLNDAQTKLLAVSGPFHYWGPVIDGHFLREPPARALKRSLWVEVDLLIGSSQDD
GLINRAKAVKQFEESQGR TSSKTA FYQALQNSLGGEDSDARVEAAATWYYSLEHSTDDYA
SFSRALENATRDYFIICPIIDMASAWAKRARGNVFMYHAPENYGHGSLELLADVQFALGL
PFYPAYEGQFSLEEKSLSLKIMQYFSHFIRSGNPNYPYEFSR KVPTFATPWPDFVPRAGG
ENYKEFSELLPNRQGLKKADCSFWSKYISSLKTSADGAKGGQSAESEEEELTAGSGLRED
LLSLQEPGSKTYSK

Figure 4 (1 of 7)

<u>10</u>	<u>20</u>	<u>30</u>	<u>40</u>	<u>50</u>	<u>60</u>
MALVLEIFTL	LASICWVSAN	IFEYQVDAQP	LRPCELQRET	AFLKQADYVP	QCAEDGSFQT
MALVLEIFTL	LASICWVSAN	IFEYQVDAQP	LRPCELQRET	AFLKQADYVP	QCAEDGSFQT
<u>70</u>	<u>80</u>	<u>90</u>	<u>100</u>	<u>110</u>	<u>120</u>
VQCQNDGRSC	WCVGANGSEV	LGSRQPGRP	ACL SFCQLQK	QQILLSGYIN	STDTSYLPQC
VQCQNDGRSC	WCVGANGSEV	LGSRQPGRP	ACL SFCQLQK	QQILLSGYIN	STDTSYLPQC
<u>130</u>	<u>140</u>	<u>150</u>	<u>160</u>	<u>170</u>	<u>180</u>
QDSGDYAPVQ	CDVQQVQCWC	VDAEGMEVYG	TRQLGRPKRC	PRSCEIRNRR	LLHGVGDKSP
QDSGDYAPVQ	CDVQQVQCWC	VDAEGMEVYG	TRQLGRPKRC	PRSCEIRNRR	LLHGVGDKSP
<u>190</u>	<u>200</u>	<u>210</u>	<u>220</u>	<u>230</u>	<u>240</u>
PQCSAEGEFM	PVQCKFVNNT	DMMIFDLVHS	YNRFPDAFVT	FSSFQRRFPE	VSGYCHCADS
PQCSAEGEFM	PVQCKFVNNT	DMMIFDLVHS	YNRFPDAFVT	FSSFQRRFPE	VSGYCHCADS
<u>250</u>	<u>260</u>	<u>270</u>	<u>280</u>	<u>290</u>	<u>300</u>
QGRELAETGL	ELLLDEIYDT	IFAGLDLPST	FTETTLYRIL	QRRFLAVQSV	ISGRFRCPTK
QGRELAETGL	ELLLDEIYDT	IFAGLDLPST	FTETTLYRIL	QRRFLAVQSV	ISGRFRCPTK
<u>310</u>	<u>320</u>	<u>330</u>	<u>340</u>	<u>350</u>	<u>360</u>
CEVERFTATS	FGHPYVPSR	RNGDYQAVQC	QTEGPCWCVD	AQGKEMHGTR	QQGEPPSCAE
CEVERFTATS	FGHPYVPSR	RNGDYQAVQC	QTEGPCWCVD	AQGKEMHGTR	QQGEPPSCAE
<u>370</u>	<u>380</u>	<u>390</u>	<u>400</u>	<u>410</u>	<u>420</u>
GQSCASERQQ	ALSRLYFGTS	GYFSQHDLFS	SPEKRWASPR	VARFATSCPP	TIKELFVDSG
GQSCASERQQ	ALSRLYFGTS	GYFSQHDLFS	SPEKRWASPR	VARFATSCPP	TIKELFVDSG
<u>430</u>	<u>440</u>	<u>450</u>	<u>460</u>	<u>470</u>	<u>480</u>
LLRPMVEGQS	QQFSVSENLL	KEAIRAIFPS	RGLARLALQF	TTNPKRLQQN	LFGGKFLVNV
LLRPMVEGQS	QQFSVSENLL	KEAIRAIFPS	RGLARLALQF	TTNPKRLQQN	LFGGKFLVNV

Figure 4 (2 of 7)

<u>490</u>	<u>500</u>	<u>510</u>	<u>520</u>	<u>530</u>	<u>540</u>
GQFNLSGALG	TRGTFNFSQF	FQQLGLASFL	NGGRQEDLAK	PLSVGLDSNS	STGTPEAAKK
GQFNLSGALG	TRGTFNFSQF	FQQLGLASFL	NGGRQEDLAK	PLSVGLDSNS	STGTPEAAKK
<u>550</u>	<u>560</u>	<u>570</u>	<u>580</u>	<u>590</u>	<u>600</u>
DGTMNKPTVG	SFGFEINLQE	NQNALKFLAS	LLELPEFLLF	LQHAI SVPED	VARDLG DVME
DGTMNKPTVG	SFGFEINLQE	NQNALKFLAS	LLELPEFLLF	LQHAI SVPED	VARDLG DVME
<u>610</u>	<u>620</u>	<u>630</u>	<u>640</u>	<u>650</u>	<u>660</u>
TVLSSQTCEQ	TPERLFVPSC	TTEGSYEDVQ	CFSGECWCVN	SWGKELPGSR	VRGGQPRCPT
TVLSSQTCEQ	TPERLFVPSC	TTEGSYEDVQ	CFSGECWCVN	SWGKELPGSR	VRGGQPRCPT
<u>670</u>	<u>680</u>	<u>690</u>	<u>700</u>	<u>710</u>	<u>720</u>
DCEKQRARMQ	SLMGSQPAGS	TLFVPACTSE	GHFLPVQCFN	SECYCVDAEG	QAIPGTRSAI
DCEKQRARMQ	SLMGSQPAGS	TLFVPACTSE	GHFLPVQCFN	SECYCVDAEG	QAIPGTRSAI
<u>730</u>	<u>740</u>	<u>750</u>	<u>760</u>	<u>770</u>	<u>780</u>
GKPKKCPTPC	QLQSEQAF LR	TVQALLSNSS	MLPTLSDTYI	PQCSTDGQWR	QVQCNGPPEQ
GKPKKCPTPC	QLQSEQAF LR	TVQALLSNSS	MLPTLSDTYI	PQCSTDGQWR	QVQCNGPPEQ
<u>790</u>	<u>800</u>	<u>810</u>	<u>820</u>	<u>830</u>	<u>840</u>
VFELYQRWEA	QNKGQDLTPA	KLLVKIMSYR	EAASGNFSLF	IQSLYEAGQQ	DVFPVLSQYP
VFELYQRWEA	QNKGQDLTPA	KLLVKIMSYR	EAASGNFSLF	IQSLYEAGQQ	DVFPVLSQYP
<u>850</u>	<u>860</u>	<u>870</u>	<u>880</u>	<u>890</u>	<u>900</u>
SLQDVPLAAL	EGKRPQPREN	ILLEPYLFWQ	ILNGQLSQYP	GSYSDFSTPL	AHFDLRNCWC
SLQDVPLAAL	EGKRPQPREN	ILLEPYLFWQ	ILNGQLSQYP	GSYSDFSTPL	AHFDLRNCWC
<u>910</u>	<u>920</u>	<u>930</u>	<u>940</u>	<u>950</u>	<u>960</u>
VDEAGQELEG	MRSEPSKLPT	CPGSCEEAKL	RVLQFIRETE	EIVSASNSSR	FPLGESFLVA
VDEAGQELEG	MRSEPSKLPT	CPGSCEEAKL	RVLQFIRETE	EIVSASNSSR	FPLGESFLVA

Figure 4 (3 of 7)

<u>970</u>	<u>980</u>	<u>990</u>	<u>1000</u>	<u>1010</u>	<u>1020</u>
KGIRLRNEDL	GLPPLFPPRE	AFAEQFLRGS	DYAIRLAAQS	TLSFYQRRRF	SPDDSAGASA
KGIRLRNEDL	GLPPLFPPRE	AFAEQFLRGS	DYAIRLAAQS	TLSFYQRRRF	SPDDSAGASA
<u>1030</u>	<u>1040</u>	<u>1050</u>	<u>1060</u>	<u>1070</u>	<u>1080</u>
LLRSGPYMPQ	CDAFGSWEFV	QCHAGTGHCW	CVDEKGGFIP	GSLTARSLQI	PQCPTTCEKS
LLRSGPYMPQ	CDAFGSWEFV	QCHAGTGHCW	CVDEKGGFIP	GSLTARSLQI	PQCPTTCEKS
<u>1090</u>	<u>1100</u>	<u>1110</u>	<u>1120</u>	<u>1130</u>	<u>1140</u>
RTSGLLSSWK	QARSQENPSP	KDLFVPACLE	TGEYARLQAS	GAGTWCVDPA	SGEELRPGSS
RTSGLLSSWK	QARSQENPSP	KDLFVPACLE	TGEYARLQAS	GAGTWCVDPA	SGEELRPGSS
<u>1150</u>	<u>1160</u>	<u>1170</u>	<u>1180</u>	<u>1190</u>	<u>1200</u>
SSAQCPSLCN	VLKSGVLSRR	VSPGYVPACR	AEDGGFSPVQ	CDQAQGSCWC	VMDSGEEVPG
SSAQCPSLCN	VLKSGVLSRR	VSPGYVPACR	AEDGGFSPVQ	CDQAQGSCWC	VMDSGEEVPG
					IPRKPI
<u>1210</u>	<u>1220</u>	<u>1230</u>	<u>1240</u>	<u>1250</u>	<u>1260</u>
TRVTGGQPAC	ESPRCPLPFN	ASEVVGGTIL	CETISGPTGS	AMQOCQLLRC	QGSWSVFPPG
TRVTGGQPAC	ESPRCPLPFN	ASEVVGGTIL	CETISGPTGS	AMQOCQLLRC	QGSWSVFPPG
SKRPVRPSLP	RSPRCPLPFN	ASEVVGGTIL	CETISGPTGS	AMQOCQLLRC	QGSWSVFPPG
<u>1270</u>	<u>1280</u>	<u>1290</u>	<u>1300</u>	<u>1310</u>	<u>1320</u>
PLICSLESGR	WESQLPQPRA	CORPQLWQTI	QTQGHFQLQL	PPGKMCSADY	ADLLQTFQVF
PLICSLESGR	WESQLPQPRA	CORPQLWQTI	QTQGHFQLQL	PPGKMCSADY	ADLLQTFQVF
PLICSLESGR	WESQLPQPRA	CORPQLWQTI	QTQGHFQLQL	PPGKMCSADY	AGLLQTFQVF
<u>1330</u>	<u>1340</u>	<u>1350</u>	<u>1360</u>	<u>1370</u>	<u>1380</u>
ILDELTARGF	CQIQVKTFGT	LVSIPVCNNS	SVQVGCLTRE	RLGVNVTWKS	RLEDIPVASL
ILDELTARGF	CQIQVKTFGT	LVSIPVCNNS	SVQVGCLTRE	RLGVNVTWKS	RLEDIPVASL
ILDELTARGF	CQIQVKTFGT	LVSIPVCNNS	SVQVGCLTRE	RLGVNVTWKS	RLEDIPVASL

Figure 4 (4 of 7)

1390	1400	1410	1420	1430	1440
PDLHDIERAL	VGKDLLGRFT	DLIQSGSFQL	HLDSKTFFPAE	TIRFLQGDHF	GTSVRTWFGC
PDLHDIERAL	VGKDLLGRFT	DLIQSGSFQL	HLDSKTFFPAE	TIRFLQGDHF	GTSVRTWFGC
PDLHDIERAL	VGKDLLGRFT	DLIQSGSFQL	HLDSKTFFPAE	TIRFLQGDHF	GTSVRTWFGC
1450	1460	1470	1480	1490	1500
SEGFYQVLTS	EASQDGLGCV	KCPEGSYSQD	EECIPCPVGF	YQEQAGSLAC	VPCPVGRTTI
SEGFYQVLTS	EASQDGLGCV	KCPEGSYSQD	EECIPCPVGF	YQEQAGSLAC	VPCPVGRTTI
SEGFYQVLTS	EASQDGLGCV	KCPEGSYSQD	EECIPCPVGF	YQEQAGSLAC	VPCPVGRTTI
1510	1520	1530	1540	1550	1560
SAGAFSQTHC	VTDCQRNEAG	LQCDQNGQYR	ASQKDRGSGK	AFCVDGEGRR	LPWWETEAPL
SAGAFSQTHL					
SAGAFSQTHC	VTDCQRNEAG	LQCDQNGQYR	ASQKDRGSGK	AFCVDGEGRR	LPWWETEAPL
1570	1580	1590	1600	1610	1620
EDSQCLMMQK	FEKVPESKVI	FDANAPVAVR	SKVPDSEFPV	MQCLTDCTED	EACSFFTVST
MQK	FEKVPESKVI	FDANAPVAVR	SKVPDSEFPV	MQCLTDCTED	EACSFFTVST
EDSQCLMMQK	FEKVPESKVI	FDANAPVAVR	SKVPDSEFPV	MQCLTDCTED	EACSFFTVST
1630	1640	1650	1660	1670	1680
TEPEISCDFY	AWTSDNVACM	TSDQKRDALG	NSKATSFGLS	RCQVKVRSYG	QDSPAVYLKK
TEPEISCDFY	AWTSDNVACM	TSDQKRDALG	NSKATSFGLS	RCQVKVRSYG	QDSPAVYLKK
TEPEISCDFY	AWTSDNVACM	TSDQKRDALG	NSKATSFGLS	RCQVKVRSYG	QDSPAVYLKK
1690	1700	1710	1720	1730	1740
GQGSTTTLQK	RFEPYGFQNM	LSGLYNPIVF	SASGANLTDA	HLFCLLACDR	DLCCDGFVLT
GQGSTTTLQK	RFEPYGFQNM	LSGLYNPIVF	SASGANLTDA	HLFCLLACDR	DLCCDGFVLT
GQGSTTTLQK	RFEPYGFQNM	LSGLYNPIVF	SASGANLTDA	HLFCLLACDR	DLCCDGFVLT
1750	1760	1770	1780	1790	1800
QVQGGAIICG	LLSSPSVLLC	NVKDWMDPSE	AWANATCPGV	TYDQESHQVI	LRLGDQEFIK
QVQGGAIICG	LLSSPSVLLC	NVKDWMDPSE	AWANATCPGV	TYDQESHQVI	LRLGDQEFIK
QVQGGAIICG	LLSSPSVLLC	NVKDWMDPSE	AWANATCPGV	TYDQESHQVI	LRLGDQEFIK

Figure 4 (5 of 7)

1810	1820	1830	1840	1850	1860
SLTPLEGTQD	TFTNFQQVYL	WKDSDMGSRP	ESMGCRKDTV	PRPASPTTEAG	LTTELFSPVD
SLTPLEGTQD	TFTNFQQVYL	WKDSDMGSRP	ESMGCRKDTV	PRPASPTTEAG	LTTELFSPVD
SLTPLEGTQD	TFTNFQQVYL	WKDSDMGSRP	ESMGCRKNTV	PRPASPTTEAG	LTTELFSPVD
1870	1880	1890	1900	1910	1920
LNQVIVNGNQ	SLSSQKHWLF	KHLFSAQQAN	LWCLSRCVQE	HSFCQLAEIT	ESASLYFTCT
LNQVIVNGNQ	SLSSQKHWLF	KHLFSAQQAN	LWCLSRCVQE	HSFCQLAEIT	ESASLYFTCT
LNQVIVNGNQ	SLSSQKHWLF	KHLFSAQQAN	LWCLSRCVQE	HSFCQLAEIT	ESASLYFTCT
1930	1940	1950	1960	1970	1980
LYPEAQVCDD	IMESNAQGCR	LILPQMPKAL	FRKKVILEDK	VKNFYTRLPF	QKLMGISIRN
LYPEAQVCDD	IMESNAQGCR	LILPQMPKAL	FRKKVILEDK	VKNFYTRLPF	QKLMGISIRN
LYPEAQVCDD	IMESNAQGCR	LILPQMPKAL	FRKKVILEDK	VKNFYTRLPF	QKLTGISIRN
1990	2000	2010	2020	2030	2040
KVPMSEKSIS	NGFFECERRC	DADPCCTGFG	FLNVSQKGG	EVTCLTLNSL	GIQMCSEENG
KVPMSEKSIS	NGFFECERRC	DADPCCTGFG	FLNVSQKGG	EVTCLTLNSL	GIQMCSEENG
KVPMSEKSIS	NGFFECERRC	DADPCCTGFG	FLNVSQKGG	EVTCLTLNSL	GIQMCSEENG
2050	2060	2070	2080	2090	2100
GAWRILDCGS	PDIEVHTYPF	GWYQKPIAQN	NAPSFCLV	LPSLTEKVSL	DSWQSLALSS
GAWRILDCGS	PDIEVHTYPF	GWYQKPIAQN	NAPSFCLV	LPSLTEKVSL	DSWQSLALSS
GAWRILDCGS	PDIEVHTYPF	GWYQKPIAQN	NAPSFCLV	LPSLTEKVSL	DSWQSLALSS
2110	2120	2130	2140	2150	2160
VVDPSIRHF	DVAHVSTAAT	SNFSAVRDLC	LSECSQHEAC	LITTLQTQPG	AVRCMFYADT
VVDPSIRHF	DVAHVSTAAT	SNFSAVRDLC	LSECSQHEAC	LITTLQTQPG	AVRCMFYADT
VVDPSIRHF	DVAHVSTAAT	SNFSAVRDLC	LSECSQHEAC	LITTLQTQPG	AVRCMFYADT

Figure 4 (6 of 7)

2170	2180	2190	2200	2210	2220
QSCTHSLQGQ	NCRLLLREEA	THIYRKPGIS	LLSYEASVPS	VPISTHGRLL	GRSQAIQVGT
QSCTHSLQGQ	NCRLLLREEA	THIYRKPGIS	LLSYEASVPS	VPISTHGRLL	GRSQAIQVGT
QSCTHSLQGQ	NCRLLLREEA	THIYRKPGIS	LLSYEASVPS	VPISTHGRLL	GRSQAIQVGT
2230	2240	2250	2260	2270	2280
SWKQVDQFLG	VPYAAPPLAE	RRFQAPEPLN	WTGSWDASKP	RASCWQPGTR	TSTSPGVSED
SWKQVDQFLG	VPYAAPPLAE	RRFQAPEPLN	WTGSWDASKP	RASCWQPGTR	TSTSPGVSED
SWKQVDQFLG	VPYAAPPLAE	RRFQAPEPLN	WTGSWDASKP	RASCWQPGTR	TSTSPGVSED
2290	2300	2310	2320	2330	2340
CLYLNVFIPQ	NVAPNASVLV	FFHNTMDREE	SEGWPAIDGS	FLAAVGNLIV	VTASYRVGVF
CLYLNVFIPQ	NVAPNASVLV	FFHNTMDREE	SEGWPAIDGS	FLAAVGNLIV	VTASYRVGVF
CLYLNVFIPQ	NVAPNASVLV	FFHNTMDREE	SEGWPAIDGS	FLAAVGNLIV	VTASYRVGVF
2350	2360	2370	2380	2390	2400
GFLSSGSGEV	SGNWGLLDQV	AALTWVQTHI	RGFGGDP RR V	SLAADRGGAD	VASIHLLTAR
GFLSSGSGEV	SGNWGLLDQV	AALTWVQTHI	RGFGGDP RR V	SLAADRGGAD	VASIHLLTAR
GFLSSGSGEV	SGNWGLLDQV	AALTWVQTHI	RGFGGDP RR V	SLAADRGGAD	VASIHLLTAR
2410	2420	2430	2440	2450	2460
ATNSQLFERRA	VLMGGSALSP	AAVISHERAQ	QQAIALAKEV	SCPMSSSQEV	VSCLRQKPAN
ATNSQLFERRA	VLMGGSALSP	AAVISHERAQ	QQAIALAKEV	SCPMSSSQEV	VSCLRQKPAN
ATNSQLFERRA	VLMGGSALSP	AAVISHERAQ	QQAIALAKEV	SCPMSSSQEV	VSCLRQKPAN
2470	2480	2490	2500	2510	2520
VLNDAQTKLL	AVSGPFHYWG	PVIDGHFLRE	PPARALKRSL	WVEVDLLIGS	SQDDGLINRA
VLNDAQTKLL	AVSGPFHYWG	PVIDGHFLRE	PPARALKRSL	WVEVDLLIGS	SQDDGLINRA
VLNDAQTKLL	AVSGPFHYWG	PVIDGHFLRE	PPARALKRSL	WVEVDLLIGS	SQDDGLINRA

Figure 4 (7 of 7)

2530	2540	2550	2560	2570	2580
KAVKQFEESR	GRTSSKTAFY	QALQNSLGGE	DSDARVEAAA	TWYYSLEHST	DDYASFSRAL
KAVKQFEESR	GRTSSKTAFY	QALQNSLGGE	DSDARVEAAA	TWYYSLEHST	DDYASFSRAL
KAVKQFEESQ	GRTSSKTAFY	QALQNSLGGE	DSDARVEAAA	TWYYSLEHST	DDYASFSRAL
2590	2600	2610	2620	2630	2640
ENATRDYFII	CPIIDMASAW	AKRARGNVFM	YHAPENYGHG	SLELLADVQF	ALGLPFYPAY
ENATRDYFII	CPIIDMASAW	AKRARGNVFM	YHAPENYGHG	SLELLADVQF	ALGLPFYPAY
ENATRDYFII	CPIIDMASAW	AKRARGNVFM	YHAPENYGHG	SLELLADVQF	ALGLPFYPAY
2650	2660	2670	2680	2690	2700
EGQFSLEEKs	LSLKIMQYFS	HFIRSGNPNY	PYEFsrKVPT	FATPWPDFVP	RAGGENYKEF
EGQFSLEEKs	LSLKIMQYFS	HFIRSGNPNY	PYEFsrKVPT	FATPWPDFVP	RAGGENYKEF
EGQFSLEEKs	LSLKIMQYFS	HFIRSGNPNY	PYEFsrKVPT	FATPWPDFVP	RAGGENYKEF
2710	2720	2730	2740	2750	2760
SELLPNRQGL	KKADCSFWSK	YISSLKTSAD	GAKGGQSAES	EEEELTAGSG	LREDLLSLQE
SELLPNRQGL	KKADCSFWSK	YISSLKTSAD	GAKGGQSAES	EEEELTAGSG	LREDLLSLQE
SELLPNRQGL	KKADCSFWSK	YISSLKTSAD	GAKGGQSAES	EEEELTAGSG	LREDLLSLQE

PGSKTYSK
PGSKTYSK
PGSKTYSK

Figure 5

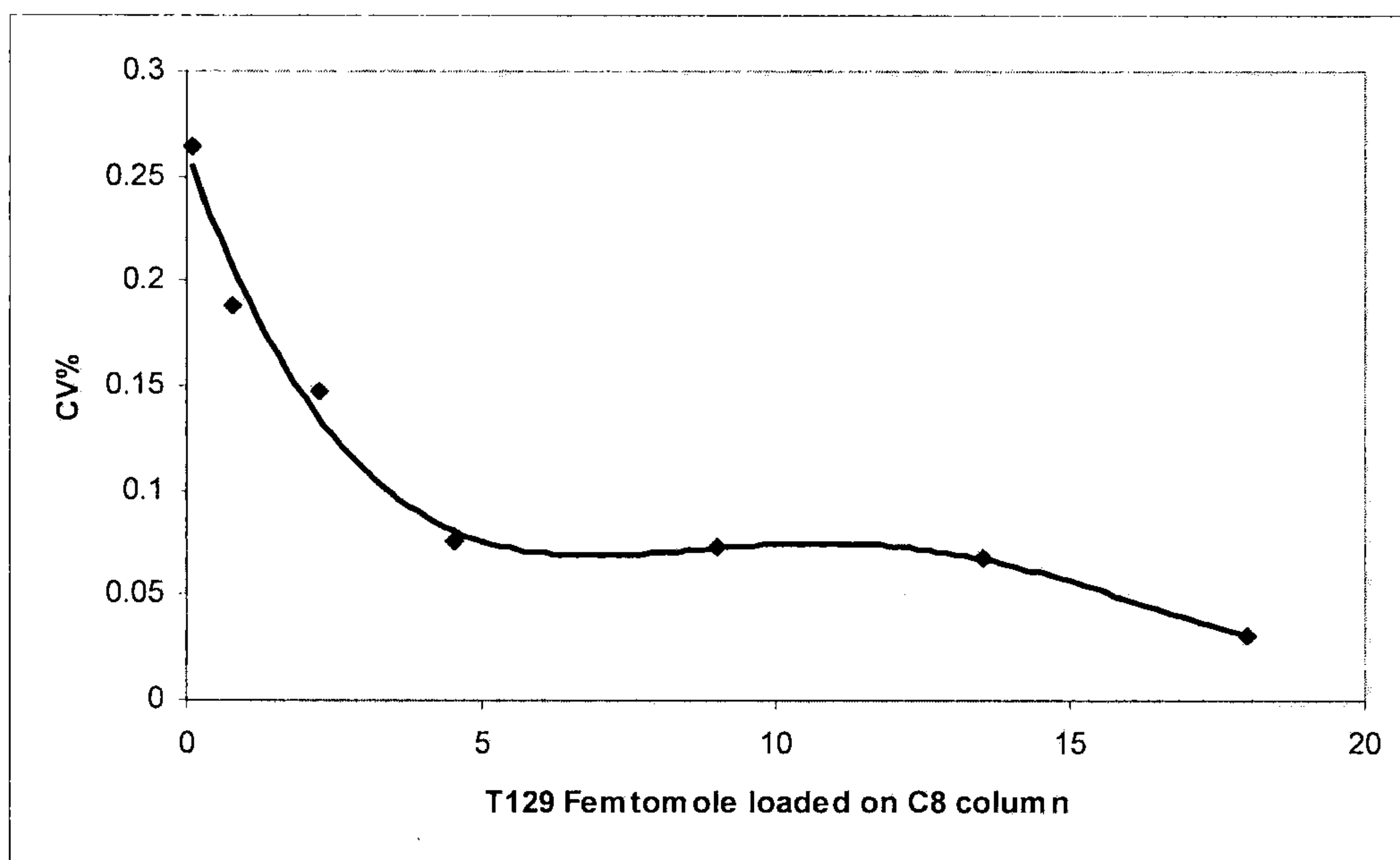


Figure 6

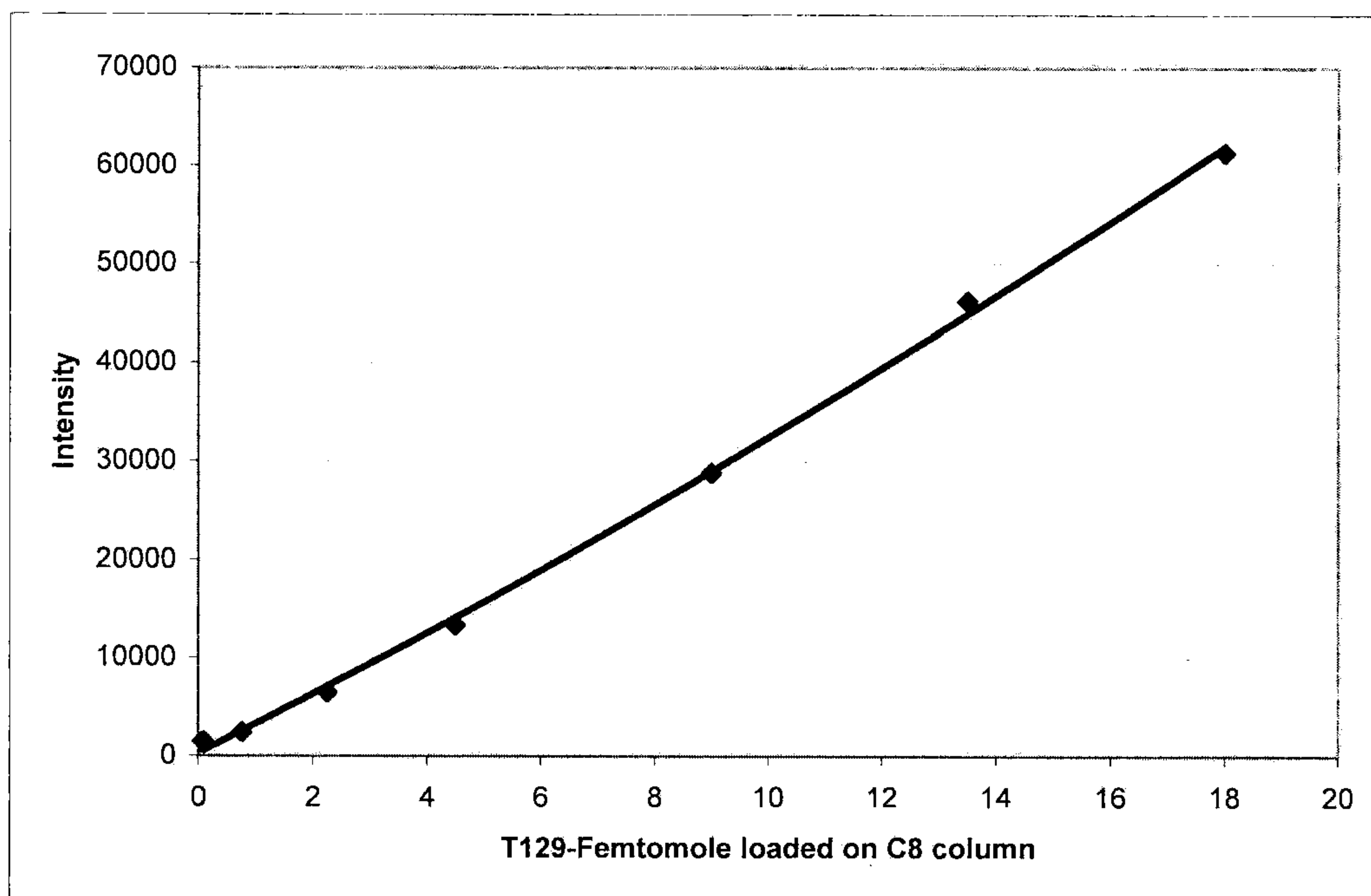


Figure 7

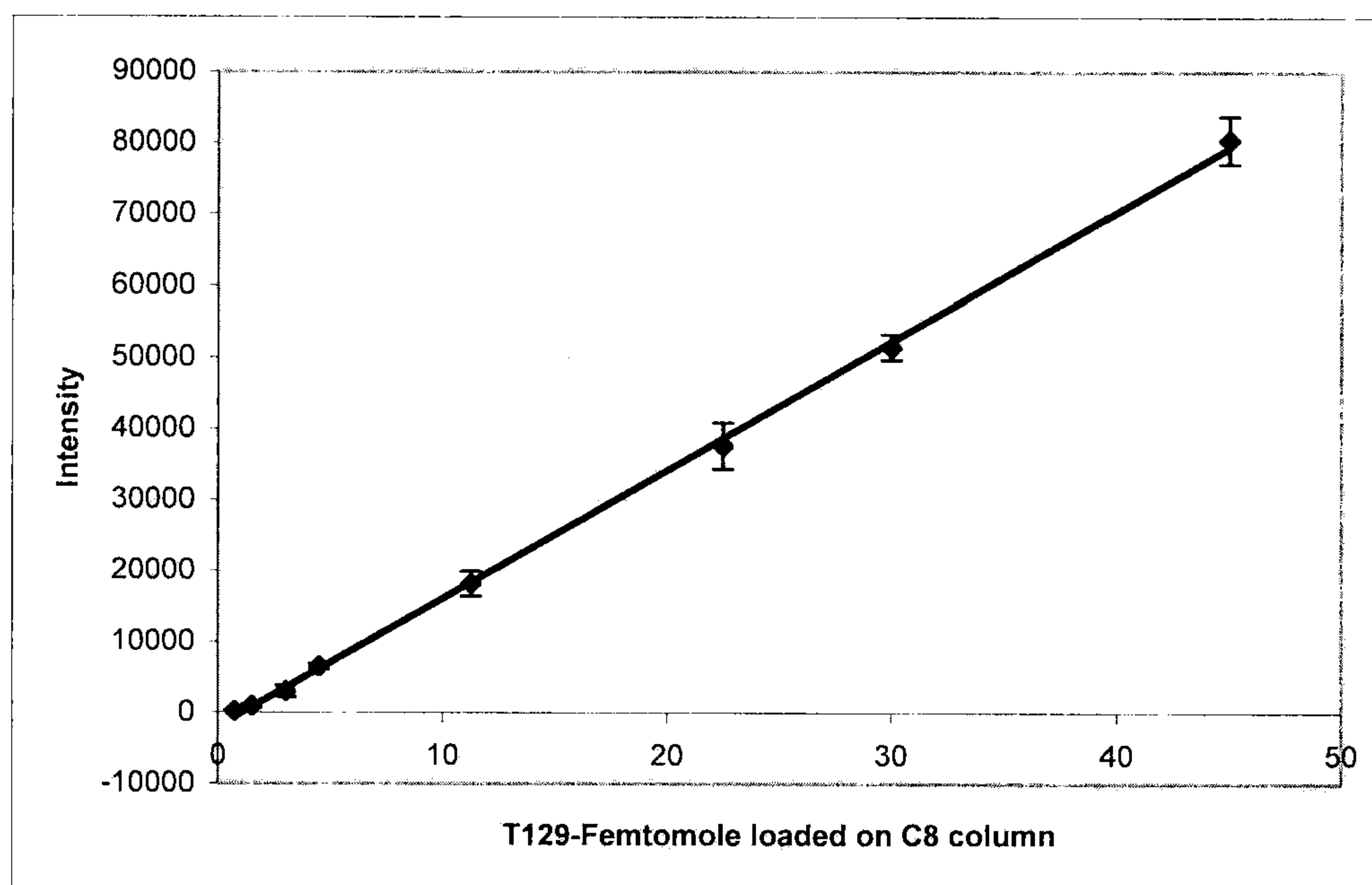


Figure 8

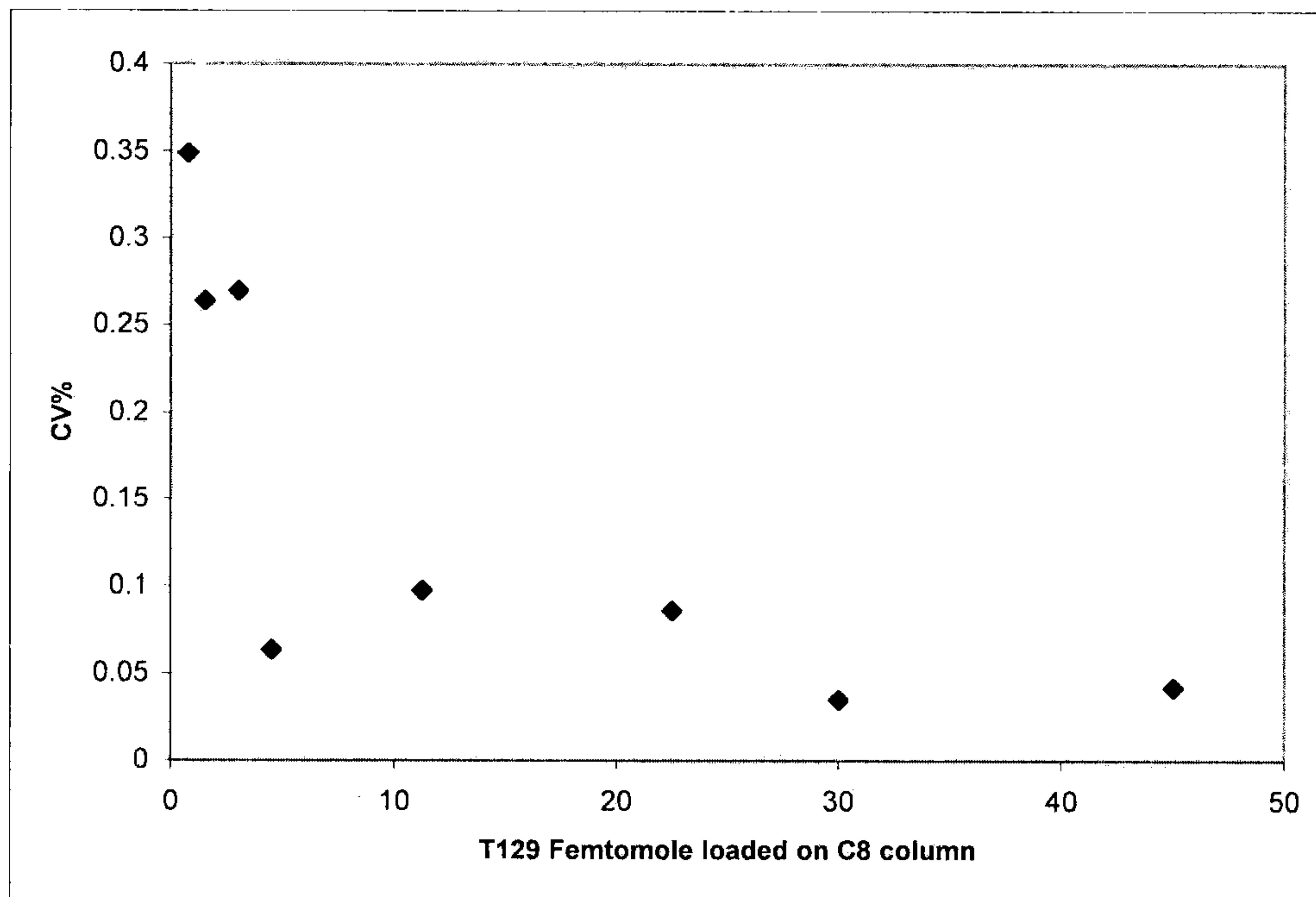
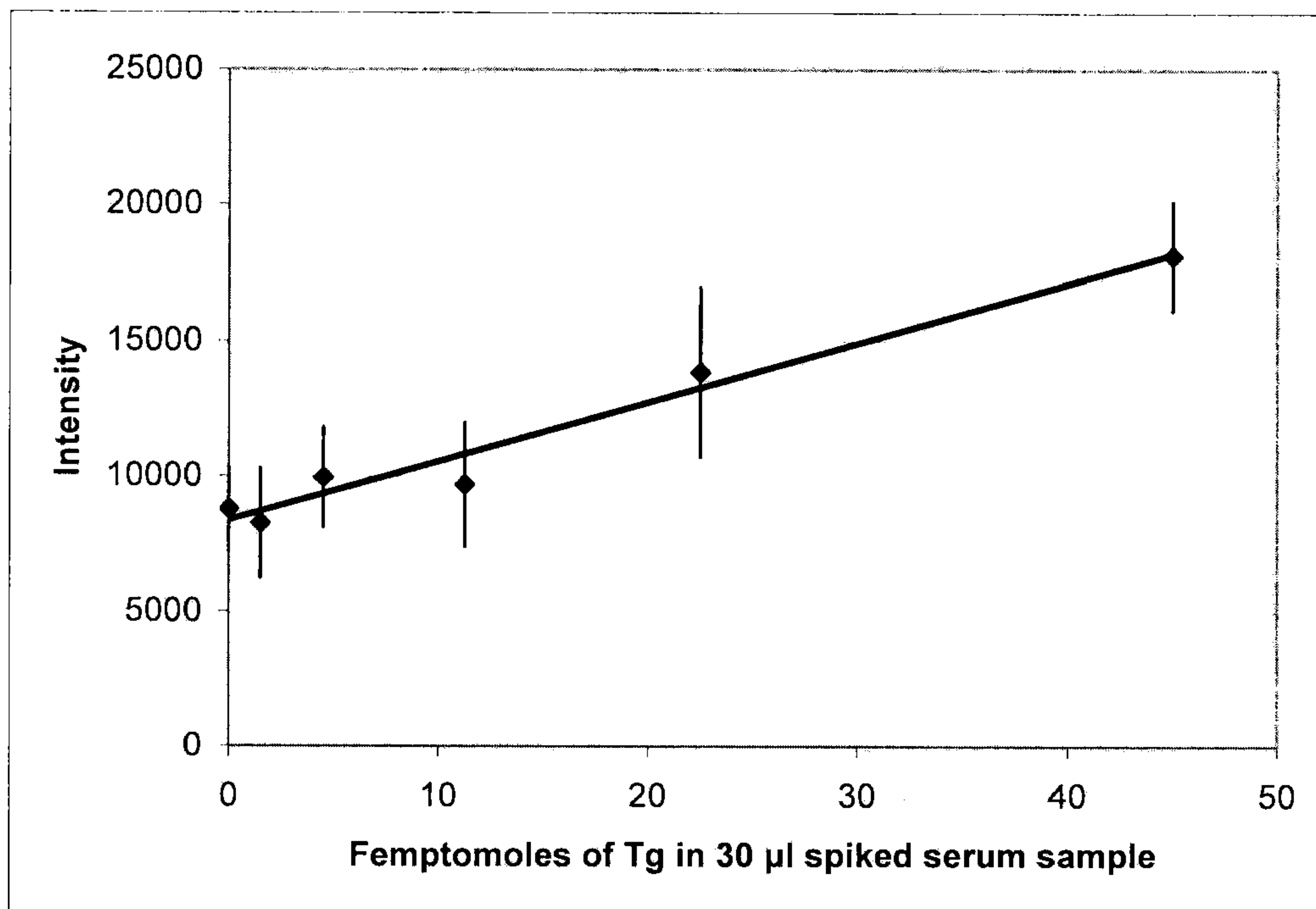


Figure 9



THYROGLOBULIN QUANTITATION BY MASS SPECTROMETRY

CROSS REFERENCE TO RELATED PATENT APPLICATIONS

This application claims priority under 35 U.S.C. §120 to U.S. application Ser. No. 12/001,076, filed Dec. 6, 2007, incorporated herein by reference in its entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Sep. 15, 2011, is named 54769284.txt and is 62,398 bytes in size.

FIELD OF THE INVENTION

The invention relates to the quantitation of thyroglobulin. In a particular aspect, the invention relates to methods for quantitation of thyroglobulin by mass spectrometry.

BACKGROUND OF THE INVENTION

The following description of the background of the invention is provided simply as an aid in understanding the invention and is not admitted to describe or constitute prior art to the invention.

Thyroglobulin, or Tg, is a large dimeric secretory glycoprotein with a molecular weight of 660 kDa comprised of noncovalently bound homodimers.

Tg molecules exist in several forms. The three major Tg molecule sequences as found in the UniProt Knowledgebase (Swiss-Prot+TrEMBL) are P01266 (Human Thyroglobulin Precursor), P01266-2 (Isoform 2 of P01266), and Q59GF02 (Human Thyroglobulin Variant). (See FIGS. 1, 2, and 3, respectively.)

P01266 is the major variant of P01266 with a length of 2768 AA; P01266-2 is an isoform of P01266 with a length of 2711 AA. P01266-2 varies from P01266 at amino acid positions 1510 to 1567 of Tg; and Q59GF0 is a thyroglobulin fragment with a length of 1574 AA. Q59GF0 contains amino acids from positions 1212 to 2768 of Tg.

Tg can only be produced in the thyroid gland and may be produced by either normal well differentiated benign thyroid cells or thyroid cancer cells. It is the precursor protein for thyroid hormone syntheses and serves as the matrix for thyroid iodine storage. Tg is used by the thyroid gland to produce the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Tg levels in the blood can be used as a tumor marker for differentiated thyroid carcinoma (DTC). A high level of Tg in the blood is not by itself an indicator of thyroid cancer, but persistence of Tg in the blood following surgical removal of the thyroid gland indicates persistence of thyroid tissue. A course of treatment following detection of Tg in the blood following surgical removal of the thyroid gland may include administration of radioiodine to ablate all remaining normal thyroid. Continued persistence of Tg in the blood following ablation of all normal thyroid could indicate that some amount of tumor is still present.

Several methods for quantitation of Tg have been developed. For example Spencer, et al., *Thyroid*, 1999, 9(5):435-41 and Persoon, et al., *Clinical Chem* 2006, 52(4):686-691 disclose immunometric, radioimmunometric, and immunochemiluminometric methods for quantitation of Tg. These

methods are all subject to methodological problems such as differences in standardization, variability in interassay sensitivity and precision, hook effects, and interference attributable to Tg antibodies. The problem of interference attributable to Tg antibodies is particularly troubling for clinical application of monitoring Tg levels as a tumor marker because up to 20% of thyroid cancer patients have Tg autoantibodies.

SUMMARY OF THE INVENTION

The present invention provides methods for quantitation of Tg in a sample by mass spectrometry, including tandem mass spectrometry.

In one aspect, methods are provided for determining the amount of Tg in a test sample that include: (a) subjecting a Tg containing test sample to digestion resulting in creation of Tg peptides; (b) purifying one or more Tg peptides; (c) ionizing one or more Tg peptides; (d) detecting the amount of the Tg peptide ion(s) by mass spectrometry; and (e) relating the amount of detected Tg peptide ion(s) to the amount of Tg in the test sample. A preferred enzyme for preparing Tg peptides is trypsin. A suitable Tg peptide for the method is one that can be evaluated by mass spectrometry and can be sufficiently purified from related peptides that may be generated from proteins other than Tg. An example of one such peptide is peptide T129 (sequence VIFDANAPVAVR) (SEQ ID NO: 4) which contains amino acids from positions 1579 to 1590 of Tg, has a molecular weight of about 1,270 Da, and is present in all three isoforms of Tg. See FIG. 4.

Formation of peptide T129 provides a unique trypsin generated peptide for thyroglobulin. Also, creation of peptide T129 from tryptic digestion of Tg should be unaffected by the presence or absence of the Tg antibodies. Thus, measurement of the increase in peptide T129 in a test sample offers a way of quantitating the amount of Tg originally in the test sample free from inference from Tg antibodies.

Any appropriate method may be used to determine the amount of Tg peptide resulting from digestion of Tg in a sample. In the event that a test sample may contain endogenous Tg peptide, steps may be taken to make certain that the endogenous peptide is not confused with peptide generated by digesting Tg in sample. One approach is to remove the endogenous Tg peptide from the sample before digesting Tg. This may be done, for example, using a size separation technique. Another approach is to analyze a portion of a test sample according to the claimed methods but excluding the digestion step in order to establish a baseline level for the endogenous peptide in the test sample. In this approach, the once a baseline is determined, it can be subtracted from the post-digestion level of the peptide, the later representing both the endogenous peptide and that generated by digestion.

Because the methods may be applied to complex test samples (particularly body fluids or test samples derived from tissue), steps may be taken to purify Tg in the test sample prior to digestion. This may be done, for example, using a size separation technique.

In some embodiments, the methods include generating one or more Tg peptide ions in which at least one of the ions has a mass/charge ratio (m/z) corresponding to that of (singly or multiply charged) peptide T129 ions. In preferred related embodiments, the methods include generating one or more Tg peptide ions in which at least one has m/z of 1272.8 ± 0.5 , 636.4 ± 0.5 , or 424.3 ± 0.5 (corresponding to singly, doubly, or triply charged peptide T129 ions). In related preferred embodiments, the methods may include generating one or more fragment ions of a Tg peptide ion in which at least one

has a m/z of 541.3 ± 0.5 , 612.3 ± 0.5 , 726.4 ± 0.5 , 797.4 ± 0.5 , 912.4 ± 0.5 , or 1059.5 ± 0.5 ; preferably one or more of the fragment ions are selected from the group consisting of ions with a m/z of 797.4 ± 0.5 , 912.4 ± 0.5 , and 1059.5 ± 0.5 .

In some embodiments, the purification in step (b) is accomplished with at least one size separation technique. Preferably, size separation techniques may be filtration, LC, or any combination thereof. In certain preferred embodiments, the test sample is a body fluid or tissue. In some embodiments, an additional step is included where a second quantity of the test sample is subjected to steps (b) through (e) in order to establish a baseline level of one or more endogenous Tg peptides. In these embodiments, this baseline level can be subtracted from the amount of Tg peptide ion(s) detected in the test sample to determine the amount of Tg peptide ion(s) that result from Tg in the original test sample. In other embodiments, the methods include an additional initial step of purifying Tg in the test sample prior to digestion. In these embodiments, the pre-digestion purification and/or the purification in step (b) may each be accomplished with at least one size separation technique. Preferably, at least one size separation technique used in both pre-digestion purification and step (b) is filtration; more preferably, this filtration is done with a molecular weight cut-off filter with molecular weight cut off that allows for retention of Tg above the filter and allows Tg peptides to pass through with the filtrate. In related embodiments, the molecular weight cut-off is about 2 kD to 300 kD; more preferably about 100 kD to 300 kD. In these embodiments, the two filtrations (pre-digestion and step (b)) may be conducted with the same filter.

In a second aspect, methods are provided for determining the amount of Tg in a test sample that include: (a) subjecting a Tg containing test sample to digestion resulting in creation of peptide T129; (b) purifying peptide T129; (c) ionizing peptide T129 to generate a precursor ion with a m/z of 636.4 ± 0.5 ; (d) fragmenting the peptide T129 precursor ion to form one or more fragment ions in which at least one has a m/z of about 797.4 ± 0.5 , 912.4 ± 0.5 , or 1059.5 ± 0.5 ; detecting the amount of peptide T129 precursor ions, one or more fragment ions, or both, by mass spectrometry; and (e) relating the amount of detected ion(s) to the amount of Tg in the test sample. In certain preferred embodiments, the test sample is a body fluid or tissue or tissue. In some embodiments, an additional step is included where a second quantity of the test sample is subjected to steps (b) through (e) in order to establish a baseline level of one or more endogenous peptide T129. In these embodiments, this baseline level can be subtracted from the amount of peptide T129 ion(s) detected in the test sample to determine the amount of peptide T129 ion(s) that result from Tg in the original test sample. In other embodiments, the methods include an additional initial step of purifying Tg in the test sample prior to digestion. In these embodiments, the pre-digestion purification and/or the purification in step (b) may each be accomplished with at least one size separation technique. Preferably, at least one size separation technique used in both pre-digestion purification and step (b) is filtration; more preferably, this filtration is done with a molecular weight cut-off filter with molecular weight cut off that allows for retention of Tg above the filter and allows Tg peptides to pass through with the filtrate. In related embodiments, the molecular weight cut-off is about 2 kD to 300 kD; more preferably about 100 kD to 300 kD. In these embodiments, the two filtrations (pre-digestion and step (b)) may be conducted with the same filter.

As used herein, the term “purification” or “purifying” does not refer to removing all materials from the sample other than the analyte(s) of interest. Instead, purification refers to a

procedure that enriches the amount of one or more analytes of interest relative to one or more other components of the sample. Purification, as used herein, does not require the isolation of an analyte from all others. In preferred embodiments, a purification step or procedure can be used to remove one or more interfering substances, e.g., one or more substances that would interfere with the operation of the instruments used in the methods or substances that may interfere with the detection of an analyte ion by mass spectrometry.

As used herein, the term “about” in reference to quantitative measurements, not including the measurement of mass of an ion, refers to the indicated value plus or minus 10%.

As used herein, the term “substantially all” refers to any proportion greater than 50%, more preferably greater than 60%, more preferably greater than 70%, more preferably greater than 80%, and more preferably greater than 90%.

As used herein, the term “test sample” refers to any sample that may contain Tg. As used herein, the term “body fluid or tissue” means any fluid or tissue that can be isolated from the body of an individual. For example, “body fluid or tissue” may include blood, plasma, serum, bile, saliva, urine, tears, perspiration, and the like. If solid tissue is to be analyzed, it may be processed to release a liquid fraction that could contain any Tg present in the tissue. The liquid fraction can then be subject to the methods described herein.

As used herein, the term “digestion” means proteolytic cleavage of proteins into peptides. Digestion agents may include trypsin, Lyc-C, Arg-R, Asp-N and the like. Digestion is carried out by adding a digestion agent (i.e., an enzyme) to a sample and incubating for some period of time.

As used herein, “Tg” or “Tg molecule” means an intact Tg protein molecule.

As used herein, the term “Tg peptide” means any peptide of 100 amino acids or less that is a fragment of the native Tg. Tg peptides can be endogenous to a test sample or formed as a result of digestion of Tg. Peptide T129 is an example of a Tg peptide formed as a result of trypsin digestion of Tg.

As used herein, the term “size separation technique” means any technique (physical or chemical) that allows for the separation of at least one species from a test sample based on any one or more of molecular weight and shape. Examples of such techniques include, but are not limited to, filtration, chromatography, and certain aspects of mass spectrometry.

As used herein, the term “chromatography” refers to a process in which a chemical mixture carried by a liquid or gas is separated into components as a result of differential distribution of the chemical entities as they flow around, over, and/or through a stationary liquid or solid phase.

As used herein, the term “liquid chromatography” or “LC” means a process of selective retardation of one or more components of a fluid solution as the fluid uniformly percolates through a column of a finely divided substance, or through capillary passageways. The retardation results from the distribution of the components of the mixture between one or more stationary phases and the bulk fluid, (i.e., mobile phase), as this fluid moves relative to the stationary phase(s). “Liquid chromatography” includes reverse phase liquid chromatography (RPLC), high performance liquid chromatography (HPLC) and high turbulence liquid chromatography (HTLC).

As used herein, the term “high performance liquid chromatography” or “HPLC” refers to liquid chromatography in which the degree of separation is increased by forcing the mobile phase under pressure through a stationary phase, typically a densely packed column.

As used herein, the term “mass spectrometry” or “MS” refers to an analytical technique to identify compounds by their mass. MS refers to methods of filtering, detecting, and

measuring ions based on their m/z . MS technology generally includes (1) ionizing the compounds to form charged species (e.g., ions); and (2) detecting the molecular weight of the ions and calculating their m/z . The compounds may be ionized and detected by any suitable means. A “mass spectrometer” generally includes an ionizer and an ion detector. In general, one or more molecules of interest are ionized, and the ions are subsequently introduced into a mass spectrographic instrument where, due to a combination of magnetic and electric fields, the ions follow a path in space that is dependent upon mass (“ m ”) and charge (“ z ”). See, e.g., U.S. Pat. Nos. 6,204,500, entitled “Mass Spectrometry From Surfaces;” 6,107,623, entitled “Methods and Apparatus for Tandem Mass Spectrometry;” 6,268,144, entitled “DNA Diagnostics Based On Mass Spectrometry;” 6,124,137, entitled “Surface-Enhanced Photolabile Attachment And Release For Desorption And Detection Of Analytes;” Wright et al., *Prostate Cancer and Prostatic Diseases* 2:264-76 (1999); and Merchant and Weinberger, *Electrophoresis* 21:1164-67 (2000).

As used herein, the term “operating in positive ion mode” refers to those mass spectrometry methods where positive ions are detected. Similarly, the term “operating in negative ion mode” refers to those mass spectrometry methods where negative ions are detected.

As used herein, the term “ionization” or “ionizing” refers to the process of generating an analyte ion having a net electrical charge equal to one or more electron units. Positive ions are those having a net positive charge of one or more electron units. Negative ions are those having a net negative charge of one or more electron units.

As used herein, the term “electron ionization” or “EI” refers to methods in which an analyte of interest in a gaseous or vapor phase interacts with a flow of electrons. Impact of the electrons with the analyte produces analyte ions, which may then be subjected to a mass spectrometry technique.

As used herein, the term “chemical ionization” or “CI” refers to methods in which a reagent gas (e.g. ammonia) is subjected to electron impact, and analyte ions are formed by the interaction of reagent gas ions and analyte molecules.

As used herein, the term “fast atom bombardment” or “FAB” refers to methods in which a beam of high energy atoms (often Xe or Ar) impacts a non-volatile sample, desorbing and ionizing molecules contained in the sample. Test samples are dissolved in a viscous liquid matrix such as glycerol, thioglycerol, *m*-nitrobenzyl alcohol, 18-crown-6 crown ether, 2-nitrophenyloctyl ether, sulfolane, diethanolamine, and triethanolamine. The choice of an appropriate matrix for a compound or sample is an empirical process.

As used herein, the term “matrix-assisted laser desorption ionization” or “MALDI” refers to methods in which a non-volatile sample is exposed to laser irradiation, which desorbs and ionizes analytes in the sample by various ionization pathways, including photo-ionization, protonation, deprotonation, and cluster decay. For MALDI, the sample is mixed with an energy-absorbing matrix, which facilitates desorption of analyte molecules.

As used herein, the term “surface enhanced laser desorption ionization” or “SELDI” refers to another method in which a non-volatile sample is exposed to laser irradiation, which desorbs and ionizes analytes in the sample by various ionization pathways, including photo-ionization, protonation, deprotonation, and cluster decay. For SELDI, the sample is typically bound to a surface that preferentially retains one or more analytes of interest. As in MALDI, this process may also employ an energy-absorbing material to facilitate ionization.

As used herein, the term “electrospray ionization” or “ESI,” refers to methods in which a solution is passed along a short length of capillary tube, to the end of which is applied a high positive or negative electric potential. Solution reaching the end of the tube is vaporized (nebulized) into a jet or spray of very small droplets of solution in solvent vapor. This mist of droplets flows through an evaporation chamber, which is heated slightly to prevent condensation and to evaporate solvent. As the droplets get smaller the electrical surface charge density increases until such time that the natural repulsion between like charges causes ions as well as neutral molecules to be released.

As used herein, the term “atmospheric pressure chemical ionization” or “APCI,” refers to mass spectrometry methods that are similar to ESI; however, APCI produces ions by ion-molecule reactions that occur within a plasma at atmospheric pressure. The plasma is maintained by an electric discharge between the spray capillary and a counter electrode. Then ions are typically extracted into the mass analyzer by use of a set of differentially pumped skimmer stages. A counterflow of dry and preheated N_2 gas may be used to improve removal of solvent. The gas-phase ionization in APCI can be more effective than ESI for analyzing less-polar species.

The term “Atmospheric Pressure Photoionization” or “APPI” as used herein refers to the form of mass spectrometry where the mechanism for the photoionization of molecule M is photon absorption and electron ejection to form the molecular M^+ . Because the photon energy typically is just above the ionization potential, the molecular ion is less susceptible to dissociation. In many cases it may be possible to analyze samples without the need for chromatography, thus saving significant time and expense. In the presence of water vapor or protic solvents, the molecular ion can extract H to form MH^+ . This tends to occur if M has a high proton affinity. This does not affect quantitation accuracy because the sum of M^+ and MH^+ is constant. Drug compounds in protic solvents are usually observed as MH^+ , whereas nonpolar compounds such as naphthalene or testosterone usually form M^+ . Robb, D. B., Covey, T. R. and Bruins, A. P. (2000): See, e.g., Robb et al., Atmospheric pressure photoionization: An ionization method for liquid chromatography-mass spectrometry. *Anal. Chem.* 72(15): 3653-3659.

As used herein, the term “inductively coupled plasma” or “ICP” refers to methods in which a sample is interacted with a partially ionized gas at a sufficiently high temperature to atomize and ionize most elements

As used, herein, the term “field desorption” refers to methods in which a non-volatile test sample is placed on an ionization surface, and an intense electric field is used to generate analyte ions.

As used herein, the term “desorption” refers to the removal of an analyte from a surface and/or the entry of an analyte into a gaseous phase.

As used herein, the term “limit of quantification” or “LOQ” refers to the point where measurements become quantitatively meaningful. The analyte response at this LOQ is identifiable, discrete and reproducible with a precision of 20% and an accuracy of 80% to 120%.

In certain preferred embodiments of the methods disclosed herein, mass spectrometry is performed in positive ion mode. In certain particularly preferred embodiments of the methods disclosed herein, mass spectrometry is performed using ESI as the method of creating ions from Tg peptides.

In preferred embodiments, the ions from Tg peptide ionization detectable in a mass spectrometer are selected from the group consisting of ions with a m/z of 636.4 ± 0.5 ,

1059.5±0.5, 921.4±0.5, 797.4±0.5, 726.4±0.5, 612.3±0.5, and 541.3±0.5; the first ion listed (m/z of 636.4±0.5) being a precursor ion with a net charge of positive 2 electron units and the latter six ions listed being fragment ions of the precursor ion. In particularly preferred embodiments, the precursor ion has a net charge of positive 2 electron units and a m/z of about 636.4±0.5, and the fragment ions have a m/z of 1059.5±0.5, 921.4±0.5, or 797.4±0.5.

In some preferred embodiments, a separately detectable internal standard peptide (e.g., T129) is introduced in the test sample after trypsin digestion. In these embodiments, all or a portion of the peptide present in the test sample both from digestion of endogenous Tg and the addition of the internal standard are ionized to produce a plurality of ions detectable in a mass spectrometer, and one or more ions produced from the peptide ionization are detected in a mass spectrometer.

In other preferred embodiments, a separately detectable internal Tg standard is provided in the test sample prior to trypsin digestion. In these embodiments, all or a portion of both the endogenous Tg and the internal standard present in the test sample are digested by trypsin resulting in formation of Tg peptides. Tg peptides are ionized to produce a plurality of ions detectable in a mass spectrometer, and one or more ions produced from Tg peptide ionization are detected by mass spectrometry.

In preferred embodiments, the ions detectable in a mass spectrometer produced from the ionization of Tg peptides resulting from Tg digestion are selected from the group consisting of ions with a m/z of 636.4±0.5, 1059.5±0.5, 921.4±0.5, 797.4±0.5, 726.4±0.5, 612.3±0.5, and 541.3±0.5; the first ion listed (m/z of 636.4±0.5) being a precursor ion with a net charge of positive 2 electron units and the latter six ions listed being fragment ions of the precursor ion. In particularly preferred embodiments, the precursor ion has a net charge of positive 2 electron units and a m/z of 636.4±0.5, and the fragment ions have a m/z of 1059.5±0.5, 921.4±0.5, 797.4±0.5.

In preferred embodiments, the presence or amount of Tg peptide ions is related to the presence or amount of Tg in the original test sample by comparison to a reference Tg sample.

In one embodiment, the methods involve the combination of LC with mass spectrometry. In another preferred embodiment, the mass spectrometry is tandem mass spectrometry (MS/MS).

The summary of the invention described above is non-limiting and other features and advantages of the invention will be apparent from the following detailed description of the invention, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the amino acid sequence for P01266 (Human Thyroglobulin Precursor) (SEQ ID NO: 1).

FIG. 2 shows the amino acid sequence for P01266-2 (Isoform 2 of P01266) (SEQ ID NO: 2).

FIG. 3 shows the amino acid sequence for Q59GF0 (Thyroglobulin Variant-Fragment) (SEQ ID NO: 3).

FIG. 4 shows a comparison of the three sequences contained in FIGS. 1-3 demonstrating that they all contain amino acids corresponding to positions 1579 to 1590 of Tg. Sequence P01266 is on top (SEQ ID NO: 1); sequence P01266-2 is in the middle (SEQ ID NO: 2); and sequence Q59GF0 is at the bottom (SEQ ID NO: 3).

FIG. 5 shows the limit of quantitation verification for Tg peptide ion with m/z corresponding to peptide T129 by MS/MS. The equation describing the trend line for FIG. 5 is

as follows: $y=1E-05x^4-0.0007x^3+0.0114x^2-0.0787x+0.2606$. $R^2=0.9833$ for this fit. Details are described in Example 1.

FIG. 6 shows the linearity of the quantitation of peptide T129 in serially diluted stock samples using an LC-MS/MS assay. The equation describing the trend line for FIG. 6 is as follows: $y=26.919x^2+2939.4x+310.78$. $R^2=0.9988$ for this fit. Details are described in Example 1.

FIG. 7 shows the limit of quantitation verification for peptide T129 in stripped serum by MS/MS. The equation describing the trend line for FIG. 7 is as follows: $y=1807.2x-1975$. $R^2=0.9993$ for this fit. Details are described in Example 2.

FIG. 8 shows the linearity of the quantitation of peptide T129 in peptide T129 spiked stripped serum using an LC-MS/MS assay. Details are described in Example 2.

FIG. 9 shows the linearity of the quantitation of Tg peptide ions with m/z corresponding to peptide T129 using an LC-MS/MS assay in stripped serum spiked with Tg prior to processing and concentration according to the methods described herein. The equation describing the trend line for FIG. 9 is as follows: $y=218.15x+8363.2$. $R^2=0.9681$ for this fit. Details are described in Example 3.

DETAILED DESCRIPTION OF THE INVENTION

Methods are described for quantitatively measuring Tg in a test sample. This quantitative measurement is achieved through the use of LC-MS/MS techniques. Prior to the use of LC-MS/MS, samples may be prepared by the following technique, or any portion thereof. A first purification of Tg in a test sample may be conducted through the use of a size separation technique such that substantially all Tg in the test sample is retained. Following the first purification step, enzymatic digestion of Tg may be carried out creating Tg peptides of interest. After digestion, another utilization of a size separation technique may be employed such that a selected Tg peptide generated in the enzymatic digestion of Tg is purified. This second size separation technique can be used to remove substantially all undigested, higher-molecular weight species. Properly executed, the sample preparation techniques ensure that selected Tg peptides quantitated by LC-MS/MS directly result from enzymatic digestion of Tg originally in the test sample; thus, the level of selected Tg peptides in the test sample at the start of LC-MS/MS is directly proportional to the amount of Tg originally present in the test sample.

Any suitable size separation technique may be utilized, but in the examples that follow, both the first and second size separation techniques are filtration through a molecular weight cut-off filter. It is also possible, as discussed in the Examples that follow, to select a molecular weight cut-off filter with an appropriate molecular weight cut-off such that the same filter can be used for both the first size separation and the second size separation.

LC, most preferably HPLC, is utilized, may be utilized either alone or in combination with other purification methods, to purify selected Tg peptides. This purification is combined with MS/MS, thereby providing an assay system for quantifying selected Tg peptides in a test sample. The quantity of the selected Tg peptides in the test sample is then used to determine the quantity of Tg in the original test sample. The Tg quantitation methods provided herein have enhanced specificity and are less subject to methodological problems (such as Tg antibody interference).

Suitable test samples may include any test sample that may contain the analyte of interest. In some preferred embodiments, a sample is a biological sample; that is, a sample

obtained from any biological source, such as an animal, a cell culture, an organ culture, and the like. In certain preferred embodiments, samples are obtained from a mammalian animal, such as a dog, cat, horse, etc. Particularly preferred mammalian animals are primates, most preferably humans. Particularly preferred samples include blood, plasma, serum, urine, saliva, tears, cerebrospinal fluid, or other body fluid or tissue samples. Such samples may be obtained, for example, from a patient; that is, a living person presenting oneself in a clinical setting for diagnosis, prognosis, or treatment of a disease or condition. The test sample is preferably obtained from a patient, for example, serum or plasma.

Sample Preparation for Mass Spectrometry

Samples may be processed or purified to obtain preparations that are suitable for analysis by mass spectrometry. Such purification will usually include chromatography, such as liquid chromatography, and may also often involve an additional purification procedure that is performed prior to chromatography. Various procedures may be used for this purpose depending on the type of sample or the type of chromatography. Examples include filtration, centrifugation, combinations thereof and the like. In certain preferred embodiments, Tg present in a test sample prior to enzymatic digestion.

Filtration is one preferred method of preparing a test sample, especially a biological test sample, such as serum or plasma, for chromatography. Such filtration is carried out by filtering a test sample through a molecular weight cut-off filter to separate species with molecular weights higher than the filter's cut-off (including Tg) from those with molecular weights lower than the filter's cut-off. The test sample remaining above the filter following complete (or near complete) filtration is substantially free of potentially interfering species with molecular weights lower than the filter's cut-off.

The pH of the test sample may then be adjusted to any point required by a digestion agent. In certain preferred embodiments, the digestion agent is trypsin and pH can be adjusted with a solution of ammonium acetate to have a pH suitable for this enzyme. In these preferred embodiments, the sample is then digested with trypsin to form Tg peptides (including peptide T129).

After trypsin digestion, the sample may be purified with a second filtration. This post-digestion filtration can be carried out similarly to the pre-digestion filtration described above (with the exception that the filtrate is retained), in order to separate Tg fragments from potentially interfering species with molecular weights higher than the filter's cut-off that may also be present in the sample. The filtrate from this post-digestion filtration can then be purified by liquid chromatography and subsequently subjected to mass spectrometry analysis.

Various methods have been described involving the use of HPLC for sample clean-up prior to mass spectrometry analysis. See, e.g., Taylor et al., *Therapeutic Drug Monitoring* 22:608-12 (2000) (manual precipitation of blood samples, followed by manual C18 solid phase extraction, injection into an HPLC for chromatography on a C18 analytical column, and MS/MS analysis); and Salm et al., *Clin. Therapeutics* 22 Supl. B:B71-B85 (2000) (manual precipitation of blood samples, followed by manual C18 solid phase extraction, injection into an HPLC for chromatography on a C18 analytical column, and MS/MS analysis). One of skill in the art may select HPLC instruments and columns that are suitable for use in the methods. The chromatographic column typically includes a medium (i.e., a packing material) to facilitate separation of chemical moieties (i.e., fractionation). The medium may include minute particles. The particles include a bonded surface that interacts with the various chemical moi-

eties to facilitate separation of the chemical moieties. One suitable bonded surface is a hydrophobic bonded surface such as an alkyl bonded surface. Alkyl bonded surfaces may include C-4, C-8, or C-18 bonded alkyl groups, preferably C-8 bonded groups. The chromatographic column includes an inlet port for receiving a sample and an outlet port for discharging an effluent that includes the fractionated sample.

In certain embodiments, an analyte may be purified by applying a sample to a column under conditions where the analyte of interest is reversibly retained by the column packing material, while one or more other materials are not retained. In these embodiments, a first mobile phase condition can be employed where the analyte of interest is retained by the column and a second mobile phase condition can subsequently be employed to remove retained material from the column, once the non-retained materials are washed through. Alternatively, an analyte may be purified by applying a sample to a column under mobile phase conditions where the analyte of interest elutes at a differential rate in comparison to one or more other materials. Such procedures may enrich the amount of one or more analytes of interest relative to one or more other components of the sample.

In one embodiment, the sample to be analyzed is applied to the column at the inlet port, eluted with a solvent or solvent mixture, and discharged at the outlet port. Different solvent modes may be selected for eluting the analytes of interest. For example, liquid chromatography may be performed using a gradient mode, an isocratic mode, or a polytypic (i.e. mixed) mode. In preferred embodiments, HPLC is performed on an analytical HPLC system with a C8 solid phase using 0.2% formic acid in HPLC Grade Ultra Pure Water and 0.2% formic acid in 100% methanol as the mobile phases.

Numerous column packings are available for chromatographic separation of samples and selection of an appropriate separation protocol is an empirical process that depends on the sample characteristics, analyte of interest, presence of interfering substances and their characteristics, etc. Commercially available HPLC columns include, but are not limited to, polar, ion exchange (both cation and anion), hydrophobic interaction, phenyl, C-2, C-8, C-18, and polar coating on porous polymer columns.

In one embodiment, the HPLC column has a C8 solid phase with a median particle size of 5 μm (nominal) and a median particle pore size of 100 \AA . In a preferred embodiment the column dimensions are 1.0 mm ID \times 50 mm length (Phenomenex Corp. Luna 5 μ C8(2) 100 \AA New Column 50 \times 1.0 mm, Phenomenex Cat. No. 00B-4249-A0 or equivalent).

During chromatography, the separation of materials is effected by variables such as choice of eluent (also known as a "mobile phase"), choice of gradient elution and the gradient conditions, temperature, etc.

Detection and Quantitation by Mass Spectrometry

In various embodiments, Tg peptides may be ionized by any method known to the skilled artisan. Mass spectrometry is performed using a mass spectrometer, which includes an ion source for ionizing the fractionated sample and creating charged molecules for further analysis. Ionization sources used in various MS techniques include, but are not limited to, electron ionization, chemical ionization, electrospray ionization (ESI), photon ionization, atmospheric pressure chemical ionization (APCI), photoionization, atmospheric pressure photoionization (APPI), fast atom bombardment (FAB)/liquid secondary ionization (LSIMS), matrix assisted laser desorption ionization (MALDI), field ionization, field desorption, thermospray/plasmaspray ionization, surface enhanced laser desorption ionization (SELDI), inductively coupled plasma (ICP) and particle beam ionization. The skilled artisan

will understand that the choice of ionization method may be determined based on the analyte to be measured, type of sample, the type of detector, the choice of positive versus negative mode, etc.

In preferred embodiments, Tg peptides are ionized by electrospray ionization (ESI) creating Tg peptide precursor ions. In related preferred embodiments, Tg peptide precursor ions are in a gaseous state and the inert collision gas is argon.

After the sample has been ionized, the positively charged ions thereby created may be analyzed to determine m/z. Suitable analyzers for determining m/z include quadrupole analyzers, ion trap analyzers, and time-of-flight analyzers. The ions may be detected using one of several detection modes. For example, only selected ions may be detected using a selective ion monitoring mode (SIM), or alternatively, multiple ions may be detected using a scanning mode, e.g., multiple reaction monitoring (MRM) or selected reaction monitoring (SRM). In preferred embodiments, ions are detected using SRM.

Preferably, m/z is determined using a quadrupole instrument. In a "quadrupole" or "quadrupole ion trap" instrument, ions in an oscillating radio frequency field experience a force proportional to the DC potential applied between electrodes, the amplitude of the RF signal, and m/z. The voltage and amplitude may be selected so that only ions having a particular m/z travel the length of the quadrupole, while all other ions are deflected. Thus, quadrupole instruments may act as both a "mass filter" and as a "mass detector" for the ions injected into the instrument.

One may enhance the resolution of the MS technique by employing "tandem mass spectrometry," or "MS/MS." In this technique, a precursor ion (also called a parent ion) generated from a molecule of interest can be filtered in an MS instrument, and the precursor ion subsequently fragmented to yield one or more fragment ions (also called daughter ions or product ions) that are then analyzed in a second MS procedure. By careful selection of precursor ions, only ions produced by certain analytes are passed to the fragmentation chamber, where collision with atoms of an inert gas produce the fragment ions. Because both the precursor and fragment ions are produced in a reproducible fashion under a given set of ionization/fragmentation conditions, the MS/MS technique may provide an extremely powerful analytical tool. For example, the combination of filtration/fragmentation may be used to eliminate interfering substances, and may be particularly useful in complex samples, such as biological samples.

Additionally, recent advances in technology, such as matrix-assisted laser desorption ionization coupled with time-of-flight analyzers ("MALDI-TOF") permit the analysis of analytes at femtomole levels in very short ion pulses. Mass spectrometers that combine time-of-flight analyzers with tandem MS are also well known to the artisan. Additionally, multiple mass spectrometry steps may be combined in methods known as "MS/MS". Various other combinations may be employed, such as MS/MS/TOF, MALDI/MS/MS/TOF, or SELDI/MS/MS/TOF mass spectrometry.

The mass spectrometer typically provides the user with an ion scan; that is, the relative abundance of each ion with a particular m/z over a given range (e.g., 400 to 1600 amu). The results of an analyte assay, that is, a mass spectrum, may be related to the amount of the analyte in the original sample by numerous methods known in the art. For example, given that sampling and analysis parameters are carefully controlled, the relative abundance of a given ion may be compared to a table that converts that relative abundance to an absolute amount of the original molecule. Alternatively, molecular standards may be run with the samples and a standard curve

constructed based on ions generated from those standards. Using such a standard curve, the relative abundance of a given ion may be converted into an absolute amount of the original molecule. In certain preferred embodiments, an internal standard is used to generate a standard curve for calculating the quantity of Tg. Methods of generating and using such standard curves are well known in the art and one of ordinary skill is capable of selecting an appropriate internal standard. Numerous other methods for relating the amount of an ion to the amount of the original molecule will be well known to those of ordinary skill in the art.

One or more steps of the methods may be performed using automated machines. In certain embodiments, one or more purification steps are performed on-line, and more preferably all of the LC purification and mass spectrometry steps may be performed in an on-line fashion.

In certain embodiments, techniques such as MS/MS are used to isolate precursor ions for further fragmentation. In these embodiments, collision activation dissociation (CAD) may be used to generate the fragment ions for further detection. In CAD, precursor ions gain energy through collisions with an inert gas, and subsequently fragment by a process referred to as "unimolecular decomposition". Sufficient energy must be deposited in the precursor ion so that certain bonds within the ion can be broken due to increased vibrational energy. In alternative embodiments, electron transfer dissociation (ETD) may be used to generate the fragment ions. In ETD, radical anions are used to transfer electrons to multiply charged peptide or protein cations resulting in random cleavage along the peptide backbone.

In particularly preferred embodiments, Tg is detected and/or quantified using LC-MS/MS as follows. A Tg peptide enriched test sample prepared as described above is subjected to LC. The flow of liquid solvent from the chromatographic column enters the heated nebulizer interface of a LC-MS/MS analyzer and the solvent/analyte mixture is converted to vapor in the heated tubing of the interface. The analyte (e.g., Tg peptides), contained in the nebulized solvent, is ionized by the corona discharge needle of the interface, which applies a large voltage to the nebulized solvent/analyte mixture. The ions (i.e. Tg peptide precursor ions) pass through the orifice of the instrument and enter the first quadrupole. Quadrupoles 1 and 3 (Q1 and Q3) are mass filters, allowing selection of ions (i.e., "precursor" and "fragment" ions) based on their m/z. Quadrupole 2 (Q2) is the collision cell, where ions are fragmented. Q1 selects for ions with m/z of peptide T129 precursor ions (m/z of 636.4±0.5). Selected precursor ions are allowed to pass into the collision chamber (Q2), while ions with any other m/z collide with the sides of Q1 and are eliminated. Precursor ions entering Q2 may be fragmented with collision activated dissociation (CAD) through collisions with neutral argon gas molecules. Alternatively, if the precursor ions entering Q2 are multiply charged cations, they may be fragmented with electron transfer dissociation (ETD). The fragment ions generated are passed into Q3, where selected fragment ions are collected while other ions are eliminated.

Using standard methods well known in the art, one of ordinary skill is capable of identifying one or more fragment ions of a particular Tg peptide precursor ion that may be used for selection in Q3. A specific fragment ion is one that will not be formed in significant amounts by other molecules with similar molecular structures. In contrast, a non-specific fragment ion is one that is formed by molecules other than the desired analyte. Suitable specific fragment ions can be identified by testing various molecular standards to determine whether fragment ions formed by a selected Tg peptide are

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also formed by other molecules with similar structures or features. Preferably, at least one fragment ion specific for Tg peptide ions with m/z corresponding to that of peptide T129 ions are identified. More preferably, one or more of these fragment ions have m/z of 797.4±0.5, 912.4±0.5 or 1059.5±0.5.

As ions collide with the detector they produce a pulse of electrons that are converted to a digital signal. The acquired data is relayed to a computer, which plots ion counts per unit time. The areas under the peaks corresponding to particular ions, or the amplitude of such peaks, are measured and the area or amplitude is correlated to the amount of the analyte of interest. In certain embodiments, the area under the curves, or amplitude of the peaks, for fragment ion(s) and/or precursor ions are measured to determine the amount of Tg peptides with m/z corresponding to peptide T129. As described above, the relative abundance of a given ion may be converted into an absolute amount of the original analyte using calibration standard curves based on peaks of one or more ions of an internal molecular standard. The absolute amount of an analyte detected by LC-MS/MS can then be converted into an absolute amount of Tg that was present in the original test sample.

The following examples serve to illustrate the invention. These examples are in no way intended to limit the scope of the methods.

EXAMPLES

Example 1

Demonstration of MS Quantitation of Peptide T129

Several samples with various known concentrations of peptide T129 were prepared by series dilution starting with a sample of known peptide T129 concentration. Peptide T129 LOQ and calibration curves were developed from LC-MS/MS analysis of these samples.

LC was performed with a Phenomenex analytical column (Phenomenex Corp. Luna 5 μ . C8(2) 100 Å New Column 50×1.0 mm). A binary HPLC eluent composed of 0.2% formic acid in ultra pure water (HPLC grade) (mobile phase A) and 0.2% formic acid in 100% methanol (mobile phase B) was applied to the analytical column to separate selected Tg peptides from other species contained in the sample. The binary eluent was applied according to the following gradient profile: as a first step, an 80/20 mixture of mobile phase A/mobile phase B was applied for 120 seconds; as a second step, a 30/70 mixture of mobile phase A/mobile phase B was applied for 60 seconds; as a third step, the relative amount of mobile phase B in the mixture was ramped to a 5/95 mixture of mobile phase A/mobile phase B over a period of 120 seconds; as a fourth step, a 5/95 mixture of mobile phase A/mobile phase B was applied for 60 seconds; as a fifth and final step, an 80/20 mixture of mobile phase A/mobile phase B was applied for 240 seconds.

The separated sample was then subjected to MS/MS for quantitation of one or more Tg peptides with m/z corresponding to peptide T129.

MS/MS was performed using a Finnigan TSQ Quantum Ultra MS/MS system (Thermo Electron Corporation). The following software programs all from ThermoElectron were used in the Examples described herein: Tune Master V 1.2 or newer, Xcalibur V 2.0 SR1 or newer, TSQ Quantum 1.4 or newer, LCQuan V 2.0 or newer, and XReport 1.0 or newer. Liquid solvent/analyte exiting the analytical HPLC column flowed to the heated nebulizer interface of a Thermo Finnigan MS/MS analyzer. The solvent/analyte mixture was converted

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to vapor in the heated tubing of the interface. Analytes in the nebulized solvent were ionized by the corona discharge needle of the interface, which applied voltage to the nebulized solvent/analyte mixture.

Ions passed to the first quadrupole (Q1), which selected ions with a m/z of 636.4±0.5. Ions entering Quadrupole 2 (Q2) collided with argon gas to generate ion fragments, which were passed to quadrupole 3 (Q3) for further selection. Mass transitions used for quantitation of precursor ions with m/z corresponding to peptide T129 during validation on positive polarity are shown in Table 1.

TABLE 1

Mass transitions for precursor ions with m/z corresponding to peptide T129 (Positive Polarity)	
Precursor Ion (m/z)	Fragment Ion (m/z)
636.4 ± 0.5	797.4 ± 0.5, 912.4 ± 0.5 & 1059.5 ± 0.5

To determine the limit of quantitation (LOQ) with a precision of 20% and an accuracy of 80% to 120%, seven different samples at varying concentrations were assayed and the reproducibility (CV) determined for each. The LOQ for one or more Tg peptides with m/z corresponding to peptide T129 was defined at about 67 amol/ μ l.

Data collected and used to develop the LOQ and Calibration curves in FIGS. 5 and 6 is shown in Table 2.

TABLE 2

Data collected and used to develop LOQ and Calibration curves for peptide T129 in spiked stripped serum samples			
Peptide T129 Concentration (Attomoles/ μ l)	Femtomoles of peptide T129 in 30 μ l sample	Average Ion Counts per Second	CV (%)
2.5	0.075	1471.6	0.264429
25	0.75	2435.6	0.188653
75	2.25	6455.4	0.147946
150	4.5	13322.4	0.075327
300	9	28805	0.073374
450	13.5	46199.6	0.067088
600	18	61302.2	0.030893

Example 2

Demonstration of Quantitation of Peptide T129 in Peptide T129 Spiked Processed, Concentrated and Digested Stripped Serum

A 500 μ l sample of stripped serum (e.g., the test sample in this Example) was added atop the filter element of a commercially available 300 kDa molecular weight cut-off filter cartridge (Pall Corp. Nanosep 300 kDa, Pall Corp. Cat. No. OD300C33).

The test sample was completely filtered upon centrifugation of the cartridge at 13 kg for 6 minutes. The filtrate was removed and discarded. 500 μ l of HPLC grade water was then added to the top of the filter and the cartridge was again centrifuged at 13 kg for 6 minutes. The filtrate was again removed and discarded. Next, 200 μ l of 20 mM ammonium acetate was added to the top of the filter. The cartridge was again centrifuged at 13 kg for 3 minutes. The filtrate was again removed and discarded and 100 μ l of 20 mM ammonium acetate was added to the top of the filter.

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Then, 15 µg of trypsin (Promega Trypsin Gold, Mass Spec Grade, Promega Corp. Cat. No. V5280 or equivalent) was added to the test sample remaining on top of the filter. The resulting mixture was incubated without removal from the filter cartridge at 37 C for up to 17 hours.

After incubation, the filter cartridge was centrifuged at 13 kg for 6 minutes, and the filtrate retained. The filter cartridge was then washed by adding 50 µl of 20 mM ammonium acetate to the top of the filter and centrifuged at 13 kg for 6 minutes. Test samples for analysis by LC-MS/MS were created by pooling the two retained post-digestion filtrates.

The starting volume of stripped serum samples subjected to the above processing and concentration was about 500 µl. The final volume of each pooled post-digestion filtrate was about 130 µl. Thus the above process concentrates samples by a factor of 3.83.

Peptide T129 was then added to the pooled post-digestion filtrates in varying concentrations. 30 µl samples were then analyzed for quantitation of peptide T129 by LC-MS/MS according to the procedure described in Example 1 with the exception that the mass transitions shown in Table 3 were used. The fragment ion with a m/z of 797.4±0.5 was not used due to increased background generated by the processed, concentrated stripped serum.

TABLE 3

Mass transitions for precursor ions with m/z corresponding to peptide T129 from peptide T129 spiked stripped serum samples (Positive Polarity)	
Precursor Ion (m/z)	Fragment Ion (m/z)
636.4 ± 0.5	912.4 ± 0.5 & 1059.5 ± 0.5

Data collected and used to develop the LOQ and Calibration curves found in FIGS. 7 and 8 is shown in Table 4.

TABLE 4

Data collected and used to develop LOQ and Calibration curves for peptide T129		
Femtomoles of Tg in spiked serum sample	Average Ion Counts per Second	CV (%)
0.75	203	0.348839
1.5	957.25	0.263782
3	2984.75	0.269659
4.5	6504.75	0.063318
11.25	18210.5	0.097296
22.5	37620	0.085823
30	51451	0.035083

Example 3

Demonstration of Quantitation of Peptide T129 in Stripped Serum Containing Various Concentrations of Added Tg

Several 500 µl samples of stripped serum containing various concentrations of added Tg were prepared according to the procedure detailed in Example 2. LC-MS/MS of the resulting test samples was carried out following the steps detailed in Example 1.

Data collected and used to develop the calibration curve found in FIG. 9 are found in Table 6.

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TABLE 6

Data collected and used to develop the calibration curve for peptide T129 MS/MS in Tg spiked stripped serum (processed and condensed as described in Example 3).		
Femtomoles of Tg in spiked serum sample	Average Ion Counts per Second	CV (%)
0	8784.667	0.176987
1.5	8259.5	0.246833
4.5	9953.25	0.186588
11.25	9696.25	0.23816
22.5	13848.25	0.225496
45	18125.5	0.110826

The contents of the articles, patents, patent applications, and all other documents and electronically available information mentioned or cited herein, are hereby incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. Applicants reserve the right to physically incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other physical and electronic documents.

The methods illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising”, “including,” “containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the invention embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the methods. This includes the generic description of the methods with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the methods are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 4

<210> SEQ ID NO 1

<211> LENGTH: 2768

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

Met Ala Leu Val Leu Glu Ile Phe Thr Leu Leu Ala Ser Ile Cys Trp
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Val Ser Ala Asn Ile Phe Glu Tyr Gln Val Asp Ala Gln Pro Leu Arg
20 25 30

Pro Cys Glu Leu Gln Arg Glu Thr Ala Phe Leu Lys Gln Ala Asp Tyr
35 40 45

Val Pro Gln Cys Ala Glu Asp Gly Ser Phe Gln Thr Val Gln Cys Gln
50 55 60

Asn Asp Gly Arg Ser Cys Trp Cys Val Gly Ala Asn Gly Ser Glu Val
65 70 75 80

Leu Gly Ser Arg Gln Pro Gly Arg Pro Val Ala Cys Leu Ser Phe Cys
85 90 95

Gln Leu Gln Lys Gln Gln Ile Leu Leu Ser Gly Tyr Ile Asn Ser Thr
100 105 110

Asp Thr Ser Tyr Leu Pro Gln Cys Gln Asp Ser Gly Asp Tyr Ala Pro
115 120 125

Val Gln Cys Asp Val Gln Gln Val Gln Cys Trp Cys Val Asp Ala Glu
130 135 140

Gly Met Glu Val Tyr Gly Thr Arg Gln Leu Gly Arg Pro Lys Arg Cys
145 150 155 160

Pro Arg Ser Cys Glu Ile Arg Asn Arg Arg Leu Leu His Gly Val Gly
165 170 175

Asp Lys Ser Pro Pro Gln Cys Ser Ala Glu Gly Glu Phe Met Pro Val
180 185 190

Gln Cys Lys Phe Val Asn Thr Thr Asp Met Met Ile Phe Asp Leu Val
195 200 205

His Ser Tyr Asn Arg Phe Pro Asp Ala Phe Val Thr Phe Ser Ser Phe
210 215 220

Gln Arg Arg Phe Pro Glu Val Ser Gly Tyr Cys His Cys Ala Asp Ser
225 230 235 240

Gln Gly Arg Glu Leu Ala Glu Thr Gly Leu Glu Leu Leu Leu Asp Glu
245 250 255

Ile Tyr Asp Thr Ile Phe Ala Gly Leu Asp Leu Pro Ser Thr Phe Thr
260 265 270

Glu Thr Thr Leu Tyr Arg Ile Leu Gln Arg Arg Phe Leu Ala Val Gln
275 280 285

Ser Val Ile Ser Gly Arg Phe Arg Cys Pro Thr Lys Cys Glu Val Glu
290 295 300

Arg Phe Thr Ala Thr Ser Phe Gly His Pro Tyr Val Pro Ser Cys Arg
305 310 315 320

Arg Asn Gly Asp Tyr Gln Ala Val Gln Cys Gln Thr Glu Gly Pro Cys
325 330 335

Trp Cys Val Asp Ala Gln Gly Lys Glu Met His Gly Thr Arg Gln Gln
340 345 350

Gly Glu Pro Pro Ser Cys Ala Glu Gly Gln Ser Cys Ala Ser Glu Arg
355 360 365

-continued

Gln Gln Ala Leu Ser Arg Leu Tyr Phe Gly Thr Ser Gly Tyr Phe Ser
 370 375 380

Gln His Asp Leu Phe Ser Ser Pro Glu Lys Arg Trp Ala Ser Pro Arg
 385 390 395 400

Val Ala Arg Phe Ala Thr Ser Cys Pro Pro Thr Ile Lys Glu Leu Phe
 405 410 415

Val Asp Ser Gly Leu Leu Arg Pro Met Val Glu Gly Gln Ser Gln Gln
 420 425 430

Phe Ser Val Ser Glu Asn Leu Leu Lys Glu Ala Ile Arg Ala Ile Phe
 435 440 445

Pro Ser Arg Gly Leu Ala Arg Leu Ala Leu Gln Phe Thr Thr Asn Pro
 450 455 460

Lys Arg Leu Gln Gln Asn Leu Phe Gly Gly Lys Phe Leu Val Asn Val
 465 470 475 480

Gly Gln Phe Asn Leu Ser Gly Ala Leu Gly Thr Arg Gly Thr Phe Asn
 485 490 495

Phe Ser Gln Phe Phe Gln Gln Leu Gly Leu Ala Ser Phe Leu Asn Gly
 500 505 510

Gly Arg Gln Glu Asp Leu Ala Lys Pro Leu Ser Val Gly Leu Asp Ser
 515 520 525

Asn Ser Ser Thr Gly Thr Pro Glu Ala Ala Lys Lys Asp Gly Thr Met
 530 535 540

Asn Lys Pro Thr Val Gly Ser Phe Gly Phe Glu Ile Asn Leu Gln Glu
 545 550 555 560

Asn Gln Asn Ala Leu Lys Phe Leu Ala Ser Leu Leu Glu Leu Pro Glu
 565 570 575

Phe Leu Leu Phe Leu Gln His Ala Ile Ser Val Pro Glu Asp Val Ala
 580 585 590

Arg Asp Leu Gly Asp Val Met Glu Thr Val Leu Ser Ser Gln Thr Cys
 595 600 605

Glu Gln Thr Pro Glu Arg Leu Phe Val Pro Ser Cys Thr Thr Glu Gly
 610 615 620

Ser Tyr Glu Asp Val Gln Cys Phe Ser Gly Glu Cys Trp Cys Val Asn
 625 630 635 640

Ser Trp Gly Lys Glu Leu Pro Gly Ser Arg Val Arg Gly Gly Gln Pro
 645 650 655

Arg Cys Pro Thr Asp Cys Glu Lys Gln Arg Ala Arg Met Gln Ser Leu
 660 665 670

Met Gly Ser Gln Pro Ala Gly Ser Thr Leu Phe Val Pro Ala Cys Thr
 675 680 685

Ser Glu Gly His Phe Leu Pro Val Gln Cys Phe Asn Ser Glu Cys Tyr
 690 695 700

Cys Val Asp Ala Glu Gly Gln Ala Ile Pro Gly Thr Arg Ser Ala Ile
 705 710 715 720

Gly Lys Pro Lys Lys Cys Pro Thr Pro Cys Gln Leu Gln Ser Glu Gln
 725 730 735

Ala Phe Leu Arg Thr Val Gln Ala Leu Leu Ser Asn Ser Ser Met Leu
 740 745 750

Pro Thr Leu Ser Asp Thr Tyr Ile Pro Gln Cys Ser Thr Asp Gly Gln
 755 760 765

Trp Arg Gln Val Gln Cys Asn Gly Pro Pro Glu Gln Val Phe Glu Leu
 770 775 780

Tyr Gln Arg Trp Glu Ala Gln Asn Lys Gly Gln Asp Leu Thr Pro Ala

-continued

785	790	795	800
Lys Leu Leu Val	Lys Ile Met Ser Tyr Arg Glu Ala Ala Ser Gly Asn		
	805	810	815
Phe Ser Leu Phe Ile Gln Ser Leu Tyr Glu Ala Gly Gln Gln Asp Val			
	820	825	830
Phe Pro Val Leu Ser Gln Tyr Pro Ser Leu Gln Asp Val Pro Leu Ala			
	835	840	845
Ala Leu Glu Gly Lys Arg Pro Gln Pro Arg Glu Asn Ile Leu Leu Glu			
	850	855	860
Pro Tyr Leu Phe Trp Gln Ile Leu Asn Gly Gln Leu Ser Gln Tyr Pro			
	865	870	875
Gly Ser Tyr Ser Asp Phe Ser Thr Pro Leu Ala His Phe Asp Leu Arg			
	885	890	895
Asn Cys Trp Cys Val Asp Glu Ala Gly Gln Glu Leu Glu Gly Met Arg			
	900	905	910
Ser Glu Pro Ser Lys Leu Pro Thr Cys Pro Gly Ser Cys Glu Glu Ala			
	915	920	925
Lys Leu Arg Val Leu Gln Phe Ile Arg Glu Thr Glu Glu Ile Val Ser			
	930	935	940
Ala Ser Asn Ser Ser Arg Phe Pro Leu Gly Glu Ser Phe Leu Val Ala			
	945	950	955
Lys Gly Ile Arg Leu Arg Asn Glu Asp Leu Gly Leu Pro Pro Leu Phe			
	965	970	975
Pro Pro Arg Glu Ala Phe Ala Glu Gln Phe Leu Arg Gly Ser Asp Tyr			
	980	985	990
Ala Ile Arg Leu Ala Ala Gln Ser Thr Leu Ser Phe Tyr Gln Arg Arg			
	995	1000	1005
Arg Phe Ser Pro Asp Asp Ser Ala Gly Ala Ser Ala Leu Leu Arg			
	1010	1015	1020
Ser Gly Pro Tyr Met Pro Gln Cys Asp Ala Phe Gly Ser Trp Glu			
	1025	1030	1035
Pro Val Gln Cys His Ala Gly Thr Gly His Cys Trp Cys Val Asp			
	1040	1045	1050
Glu Lys Gly Gly Phe Ile Pro Gly Ser Leu Thr Ala Arg Ser Leu			
	1055	1060	1065
Gln Ile Pro Gln Cys Pro Thr Thr Cys Glu Lys Ser Arg Thr Ser			
	1070	1075	1080
Gly Leu Leu Ser Ser Trp Lys Gln Ala Arg Ser Gln Glu Asn Pro			
	1085	1090	1095
Ser Pro Lys Asp Leu Phe Val Pro Ala Cys Leu Glu Thr Gly Glu			
	1100	1105	1110
Tyr Ala Arg Leu Gln Ala Ser Gly Ala Gly Thr Trp Cys Val Asp			
	1115	1120	1125
Pro Ala Ser Gly Glu Glu Leu Arg Pro Gly Ser Ser Ser Ser Ala			
	1130	1135	1140
Gln Cys Pro Ser Leu Cys Asn Val Leu Lys Ser Gly Val Leu Ser			
	1145	1150	1155
Arg Arg Val Ser Pro Gly Tyr Val Pro Ala Cys Arg Ala Glu Asp			
	1160	1165	1170
Gly Gly Phe Ser Pro Val Gln Cys Asp Gln Ala Gln Gly Ser Cys			
	1175	1180	1185
Trp Cys Val Met Asp Ser Gly Glu Glu Val Pro Gly Thr Arg Val			
	1190	1195	1200

-continued

Thr	Gly	Gly	Gln	Pro	Ala	Cys	Glu	Ser	Pro	Arg	Cys	Pro	Leu	Pro
1205						1210					1215			
Phe	Asn	Ala	Ser	Glu	Val	Val	Gly	Gly	Thr	Ile	Leu	Cys	Glu	Thr
1220						1225					1230			
Ile	Ser	Gly	Pro	Thr	Gly	Ser	Ala	Met	Gln	Gln	Cys	Gln	Leu	Leu
1235						1240					1245			
Cys	Arg	Gln	Gly	Ser	Trp	Ser	Val	Phe	Pro	Pro	Gly	Pro	Leu	Ile
1250						1255					1260			
Cys	Ser	Leu	Glu	Ser	Gly	Arg	Trp	Glu	Ser	Gln	Leu	Pro	Gln	Pro
1265						1270					1275			
Arg	Ala	Cys	Gln	Arg	Pro	Gln	Leu	Trp	Gln	Thr	Ile	Gln	Thr	Gln
1280						1285					1290			
Gly	His	Phe	Gln	Leu	Gln	Leu	Pro	Pro	Gly	Lys	Met	Cys	Ser	Ala
1295						1300					1305			
Asp	Tyr	Ala	Asp	Leu	Leu	Gln	Thr	Phe	Gln	Val	Phe	Ile	Leu	Asp
1310						1315					1320			
Glu	Leu	Thr	Ala	Arg	Gly	Phe	Cys	Gln	Ile	Gln	Val	Lys	Thr	Phe
1325						1330					1335			
Gly	Thr	Leu	Val	Ser	Ile	Pro	Val	Cys	Asn	Asn	Ser	Ser	Val	Gln
1340						1345					1350			
Val	Gly	Cys	Leu	Thr	Arg	Glu	Arg	Leu	Gly	Val	Asn	Val	Thr	Trp
1355						1360					1365			
Lys	Ser	Arg	Leu	Glu	Asp	Ile	Pro	Val	Ala	Ser	Leu	Pro	Asp	Leu
1370						1375					1380			
His	Asp	Ile	Glu	Arg	Ala	Leu	Val	Gly	Lys	Asp	Leu	Leu	Gly	Arg
1385						1390					1395			
Phe	Thr	Asp	Leu	Ile	Gln	Ser	Gly	Ser	Phe	Gln	Leu	His	Leu	Asp
1400						1405					1410			
Ser	Lys	Thr	Phe	Pro	Ala	Glu	Thr	Ile	Arg	Phe	Leu	Gln	Gly	Asp
1415						1420					1425			
His	Phe	Gly	Thr	Ser	Pro	Arg	Thr	Trp	Phe	Gly	Cys	Ser	Glu	Gly
1430						1435					1440			
Phe	Tyr	Gln	Val	Leu	Thr	Ser	Glu	Ala	Ser	Gln	Asp	Gly	Leu	Gly
1445						1450					1455			
Cys	Val	Lys	Cys	Pro	Glu	Gly	Ser	Tyr	Ser	Gln	Asp	Glu	Glu	Cys
1460						1465					1470			
Ile	Pro	Cys	Pro	Val	Gly	Phe	Tyr	Gln	Glu	Gln	Ala	Gly	Ser	Leu
1475						1480					1485			
Ala	Cys	Val	Pro	Cys	Pro	Val	Gly	Arg	Thr	Thr	Ile	Ser	Ala	Gly
1490						1495					1500			
Ala	Phe	Ser	Gln	Thr	His	Cys	Val	Thr	Asp	Cys	Gln	Arg	Asn	Glu
1505						1510					1515			
Ala	Gly	Leu	Gln	Cys	Asp	Gln	Asn	Gly	Gln	Tyr	Arg	Ala	Ser	Gln
1520						1525					1530			
Lys	Asp	Arg	Gly	Ser	Gly	Lys	Ala	Phe	Cys	Val	Asp	Gly	Glu	Gly
1535						1540					1545			
Arg	Arg	Leu	Pro	Trp	Trp	Glu	Thr	Glu	Ala	Pro	Leu	Glu	Asp	Ser
1550						1555					1560			
Gln	Cys	Leu	Met	Met	Gln	Lys	Phe	Glu	Lys	Val	Pro	Glu	Ser	Lys
1565						1570					1575			
Val	Ile	Phe	Asp	Ala	Asn	Ala	Pro	Val	Ala	Val	Arg	Ser	Lys	Val
1580						1585					1590			
Pro	Asp	Ser	Glu	Phe	Pro	Val	Met	Gln	Cys	Leu	Thr	Asp	Cys	Thr
1595						1600					1605			

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Glu Asp 1610	Glu Ala Cys Ser 1610	Phe Phe Thr Val Ser Thr 1615 1620	Thr Thr Glu Pro
Glu Ile 1625	Ser Cys Asp Phe Tyr 1630	Ala Trp Thr Ser Asp 1635	Asn Val Ala
Cys Met 1640	Thr Ser Asp Gln Lys 1645	Arg Asp Ala Leu Gly 1650	Asn Ser Lys
Ala Thr 1655	Ser Phe Gly Ser Leu 1660	Arg Cys Gln Val Lys 1665	Val Arg Ser
His Gly 1670	Gln Asp Ser Pro Ala 1675	Val Tyr Leu Lys Lys 1680	Gly Gln Gly
Ser Thr 1685	Thr Thr Leu Gln Lys 1690	Arg Phe Glu Pro Thr 1695	Gly Phe Gln
Asn Met 1700	Leu Ser Gly Leu Tyr 1705	Asn Pro Ile Val Phe 1710	Ser Ala Ser
Gly Ala 1715	Asn Leu Thr Asp Ala 1720	His Leu Phe Cys Leu 1725	Leu Ala Cys
Asp Arg 1730	Asp Leu Cys Cys Asp 1735	Gly Phe Val Leu Thr 1740	Gln Val Gln
Gly Gly 1745	Ala Ile Ile Cys Gly 1750	Leu Leu Ser Ser Pro 1755	Ser Val Leu
Leu Cys 1760	Asn Val Lys Asp Trp 1765	Met Asp Pro Ser Glu 1770	Ala Trp Ala
Asn Ala 1775	Thr Cys Pro Gly Val 1780	Thr Tyr Asp Gln Glu 1785	Ser His Gln
Val Ile 1790	Leu Arg Leu Gly Asp 1795	Gln Glu Phe Ile Lys 1800	Ser Leu Thr
Pro Leu 1805	Glu Gly Thr Gln Asp 1810	Thr Phe Thr Asn Phe 1815	Gln Gln Val
Tyr Leu 1820	Trp Lys Asp Ser Asp 1825	Met Gly Ser Arg Pro 1830	Glu Ser Met
Gly Cys 1835	Arg Lys Asp Thr Val 1840	Pro Arg Pro Ala Ser 1845	Pro Thr Glu
Ala Gly 1850	Leu Thr Thr Glu Leu 1855	Phe Ser Pro Val Asp 1860	Leu Asn Gln
Val Ile 1865	Val Asn Gly Asn Gln 1870	Ser Leu Ser Ser Gln 1875	Lys His Trp
Leu Phe 1880	Lys His Leu Phe Ser 1885	Ala Gln Gln Ala Asn 1890	Leu Trp Cys
Leu Ser 1895	Arg Cys Val Gln Glu 1900	His Ser Phe Cys Gln 1905	Leu Ala Glu
Ile Thr 1910	Glu Ser Ala Ser Leu 1915	Tyr Phe Thr Cys Thr 1920	Leu Tyr Pro
Glu Ala 1925	Gln Val Cys Asp Asp 1930	Ile Met Glu Ser Asn 1935	Ala Gln Gly
Cys Arg 1940	Leu Ile Leu Pro Gln 1945	Met Pro Lys Ala Leu 1950	Phe Arg Lys
Lys Val 1955	Ile Leu Glu Asp Lys 1960	Val Lys Asn Phe Tyr 1965	Thr Arg Leu
Pro Phe 1970	Gln Lys Leu Met Gly 1975	Ile Ser Ile Arg Asn 1980	Lys Val Pro
Met Ser 1985	Glu Lys Ser Ile Ser 1990	Asn Gly Phe Phe Glu 1995	Cys Glu Arg
Arg Cys	Asp Ala Asp Pro Cys	Cys Thr Gly Phe Gly	Phe Leu Asn

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2000		2005		2010										
Val	Ser	Gln	Leu	Lys	Gly	Gly	Glu	Val	Thr	Cys	Leu	Thr	Leu	Asn
2015						2020					2025			
Ser	Leu	Gly	Ile	Gln	Met	Cys	Ser	Glu	Glu	Asn	Gly	Gly	Ala	Trp
2030						2035					2040			
Arg	Ile	Leu	Asp	Cys	Gly	Ser	Pro	Asp	Ile	Glu	Val	His	Thr	Tyr
2045						2050					2055			
Pro	Phe	Gly	Trp	Tyr	Gln	Lys	Pro	Ile	Ala	Gln	Asn	Asn	Ala	Pro
2060						2065					2070			
Ser	Phe	Cys	Pro	Leu	Val	Val	Leu	Pro	Ser	Leu	Thr	Glu	Lys	Val
2075						2080					2085			
Ser	Leu	Asp	Ser	Trp	Gln	Ser	Leu	Ala	Leu	Ser	Ser	Val	Val	Val
2090						2095					2100			
Asp	Pro	Ser	Ile	Arg	His	Phe	Asp	Val	Ala	His	Val	Ser	Thr	Ala
2105						2110					2115			
Ala	Thr	Ser	Asn	Phe	Ser	Ala	Val	Arg	Asp	Leu	Cys	Leu	Ser	Glu
2120						2125					2130			
Cys	Ser	Gln	His	Glu	Ala	Cys	Leu	Ile	Thr	Thr	Leu	Gln	Thr	Gln
2135						2140					2145			
Pro	Gly	Ala	Val	Arg	Cys	Met	Phe	Tyr	Ala	Asp	Thr	Gln	Ser	Cys
2150						2155					2160			
Thr	His	Ser	Leu	Gln	Gly	Gln	Asn	Cys	Arg	Leu	Leu	Leu	Arg	Glu
2165						2170					2175			
Glu	Ala	Thr	His	Ile	Tyr	Arg	Lys	Pro	Gly	Ile	Ser	Leu	Leu	Ser
2180						2185					2190			
Tyr	Glu	Ala	Ser	Val	Pro	Ser	Val	Pro	Ile	Ser	Thr	His	Gly	Arg
2195						2200					2205			
Leu	Leu	Gly	Arg	Ser	Gln	Ala	Ile	Gln	Val	Gly	Thr	Ser	Trp	Lys
2210						2215					2220			
Gln	Val	Asp	Gln	Phe	Leu	Gly	Val	Pro	Tyr	Ala	Ala	Pro	Pro	Leu
2225						2230					2235			
Ala	Glu	Arg	Arg	Phe	Gln	Ala	Pro	Glu	Pro	Leu	Asn	Trp	Thr	Gly
2240						2245					2250			
Ser	Trp	Asp	Ala	Ser	Lys	Pro	Arg	Ala	Ser	Cys	Trp	Gln	Pro	Gly
2255						2260					2265			
Thr	Arg	Thr	Ser	Thr	Ser	Pro	Gly	Val	Ser	Glu	Asp	Cys	Leu	Tyr
2270						2275					2280			
Leu	Asn	Val	Phe	Ile	Pro	Gln	Asn	Val	Ala	Pro	Asn	Ala	Ser	Val
2285						2290					2295			
Leu	Val	Phe	Phe	His	Asn	Thr	Met	Asp	Arg	Glu	Glu	Ser	Glu	Gly
2300						2305					2310			
Trp	Pro	Ala	Ile	Asp	Gly	Ser	Phe	Leu	Ala	Ala	Val	Gly	Asn	Leu
2315						2320					2325			
Ile	Val	Val	Thr	Ala	Ser	Tyr	Arg	Val	Gly	Val	Phe	Gly	Phe	Leu
2330						2335					2340			
Ser	Ser	Gly	Ser	Gly	Glu	Val	Ser	Gly	Asn	Trp	Gly	Leu	Leu	Asp
2345						2350					2355			
Gln	Val	Ala	Ala	Leu	Thr	Trp	Val	Gln	Thr	His	Ile	Arg	Gly	Phe
2360						2365					2370			
Gly	Gly	Asp	Pro	Arg	Arg	Val	Ser	Leu	Ala	Ala	Asp	Arg	Gly	Gly
2375						2380					2385			
Ala	Asp	Val	Ala	Ser	Ile	His	Leu	Leu	Thr	Ala	Arg	Ala	Thr	Asn
2390						2395					2400			

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Ser	Gln	Leu	Phe	Arg	Arg	Ala	Val	Leu	Met	Gly	Gly	Ser	Ala	Leu
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2420						2425					2430			
Ile	Ala	Leu	Ala	Lys	Glu	Val	Ser	Cys	Pro	Met	Ser	Ser	Ser	Gln
2435						2440					2445			
Glu	Val	Val	Ser	Cys	Leu	Arg	Gln	Lys	Pro	Ala	Asn	Val	Leu	Asn
2450						2455					2460			
Asp	Ala	Gln	Thr	Lys	Leu	Leu	Ala	Val	Ser	Gly	Pro	Phe	His	Tyr
2465						2470					2475			
Trp	Gly	Pro	Val	Ile	Asp	Gly	His	Phe	Leu	Arg	Glu	Pro	Pro	Ala
2480						2485					2490			
Arg	Ala	Leu	Lys	Arg	Ser	Leu	Trp	Val	Glu	Val	Asp	Leu	Leu	Ile
2495						2500					2505			
Gly	Ser	Ser	Gln	Asp	Asp	Gly	Leu	Ile	Asn	Arg	Ala	Lys	Ala	Val
2510						2515					2520			
Lys	Gln	Phe	Glu	Glu	Ser	Arg	Gly	Arg	Thr	Ser	Ser	Lys	Thr	Ala
2525						2530					2535			
Phe	Tyr	Gln	Ala	Leu	Gln	Asn	Ser	Leu	Gly	Gly	Glu	Asp	Ser	Asp
2540						2545					2550			
Ala	Arg	Val	Glu	Ala	Ala	Ala	Thr	Trp	Tyr	Tyr	Ser	Leu	Glu	His
2555						2560					2565			
Ser	Thr	Asp	Asp	Tyr	Ala	Ser	Phe	Ser	Arg	Ala	Leu	Glu	Asn	Ala
2570						2575					2580			
Thr	Arg	Asp	Tyr	Phe	Ile	Ile	Cys	Pro	Ile	Ile	Asp	Met	Ala	Ser
2585						2590					2595			
Ala	Trp	Ala	Lys	Arg	Ala	Arg	Gly	Asn	Val	Phe	Met	Tyr	His	Ala
2600						2605					2610			
Pro	Glu	Asn	Tyr	Gly	His	Gly	Ser	Leu	Glu	Leu	Leu	Ala	Asp	Val
2615						2620					2625			
Gln	Phe	Ala	Leu	Gly	Leu	Pro	Phe	Tyr	Pro	Ala	Tyr	Glu	Gly	Gln
2630						2635					2640			
Phe	Ser	Leu	Glu	Glu	Lys	Ser	Leu	Ser	Leu	Lys	Ile	Met	Gln	Tyr
2645						2650					2655			
Phe	Ser	His	Phe	Ile	Arg	Ser	Gly	Asn	Pro	Asn	Tyr	Pro	Tyr	Glu
2660						2665					2670			
Phe	Ser	Arg	Lys	Val	Pro	Thr	Phe	Ala	Thr	Pro	Trp	Pro	Asp	Phe
2675						2680					2685			
Val	Pro	Arg	Ala	Gly	Gly	Glu	Asn	Tyr	Lys	Glu	Phe	Ser	Glu	Leu
2690						2695					2700			
Leu	Pro	Asn	Arg	Gln	Gly	Leu	Lys	Lys	Ala	Asp	Cys	Ser	Phe	Trp
2705						2710					2715			
Ser	Lys	Tyr	Ile	Ser	Ser	Leu	Lys	Thr	Ser	Ala	Asp	Gly	Ala	Lys
2720						2725					2730			
Gly	Gly	Gln	Ser	Ala	Glu	Ser	Glu	Glu	Glu	Glu	Leu	Thr	Ala	Gly
2735						2740					2745			
Ser	Gly	Leu	Arg	Glu	Asp	Leu	Leu	Ser	Leu	Gln	Glu	Pro	Gly	Ser
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Lys	Thr	Tyr	Ser	Lys										
2765														

<210> SEQ ID NO 2

<211> LENGTH: 2711

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 2

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 20 25 30
 Pro Cys Glu Leu Gln Arg Glu Thr Ala Phe Leu Lys Gln Ala Asp Tyr
 35 40 45
 Val Pro Gln Cys Ala Glu Asp Gly Ser Phe Gln Thr Val Gln Cys Gln
 50 55 60
 Asn Asp Gly Arg Ser Cys Trp Cys Val Gly Ala Asn Gly Ser Glu Val
 65 70 75 80
 Leu Gly Ser Arg Gln Pro Gly Arg Pro Val Ala Cys Leu Ser Phe Cys
 85 90 95
 Gln Leu Gln Lys Gln Gln Ile Leu Leu Ser Gly Tyr Ile Asn Ser Thr
 100 105 110
 Asp Thr Ser Tyr Leu Pro Gln Cys Gln Asp Ser Gly Asp Tyr Ala Pro
 115 120 125
 Val Gln Cys Asp Val Gln Gln Val Gln Cys Trp Cys Val Asp Ala Glu
 130 135 140
 Gly Met Glu Val Tyr Gly Thr Arg Gln Leu Gly Arg Pro Lys Arg Cys
 145 150 155 160
 Pro Arg Ser Cys Glu Ile Arg Asn Arg Arg Leu Leu His Gly Val Gly
 165 170 175
 Asp Lys Ser Pro Pro Gln Cys Ser Ala Glu Gly Glu Phe Met Pro Val
 180 185 190
 Gln Cys Lys Phe Val Asn Thr Thr Asp Met Met Ile Phe Asp Leu Val
 195 200 205
 His Ser Tyr Asn Arg Phe Pro Asp Ala Phe Val Thr Phe Ser Ser Phe
 210 215 220
 Gln Arg Arg Phe Pro Glu Val Ser Gly Tyr Cys His Cys Ala Asp Ser
 225 230 235 240
 Gln Gly Arg Glu Leu Ala Glu Thr Gly Leu Glu Leu Leu Leu Asp Glu
 245 250 255
 Ile Tyr Asp Thr Ile Phe Ala Gly Leu Asp Leu Pro Ser Thr Phe Thr
 260 265 270
 Glu Thr Thr Leu Tyr Arg Ile Leu Gln Arg Arg Phe Leu Ala Val Gln
 275 280 285
 Ser Val Ile Ser Gly Arg Phe Arg Cys Pro Thr Lys Cys Glu Val Glu
 290 295 300
 Arg Phe Thr Ala Thr Ser Phe Gly His Pro Tyr Val Pro Ser Cys Arg
 305 310 315 320
 Arg Asn Gly Asp Tyr Gln Ala Val Gln Cys Gln Thr Glu Gly Pro Cys
 325 330 335
 Trp Cys Val Asp Ala Gln Gly Lys Glu Met His Gly Thr Arg Gln Gln
 340 345 350
 Gly Glu Pro Pro Ser Cys Ala Glu Gly Gln Ser Cys Ala Ser Glu Arg
 355 360 365
 Gln Gln Ala Leu Ser Arg Leu Tyr Phe Gly Thr Ser Gly Tyr Phe Ser
 370 375 380
 Gln His Asp Leu Phe Ser Ser Pro Glu Lys Arg Trp Ala Ser Pro Arg
 385 390 395 400
 Val Ala Arg Phe Ala Thr Ser Cys Pro Pro Thr Ile Lys Glu Leu Phe
 405 410 415

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Val Asp Ser Gly Leu Leu Arg Pro Met Val Glu Gly Gln Ser Gln Gln
 420 425 430
 Phe Ser Val Ser Glu Asn Leu Leu Lys Glu Ala Ile Arg Ala Ile Phe
 435 440 445
 Pro Ser Arg Gly Leu Ala Arg Leu Ala Leu Gln Phe Thr Thr Asn Pro
 450 455 460
 Lys Arg Leu Gln Gln Asn Leu Phe Gly Gly Lys Phe Leu Val Asn Val
 465 470 475 480
 Gly Gln Phe Asn Leu Ser Gly Ala Leu Gly Thr Arg Gly Thr Phe Asn
 485 490 495
 Phe Ser Gln Phe Phe Gln Gln Leu Gly Leu Ala Ser Phe Leu Asn Gly
 500 505 510
 Gly Arg Gln Glu Asp Leu Ala Lys Pro Leu Ser Val Gly Leu Asp Ser
 515 520 525
 Asn Ser Ser Thr Gly Thr Pro Glu Ala Ala Lys Lys Asp Gly Thr Met
 530 535 540
 Asn Lys Pro Thr Val Gly Ser Phe Gly Phe Glu Ile Asn Leu Gln Glu
 545 550 555 560
 Asn Gln Asn Ala Leu Lys Phe Leu Ala Ser Leu Leu Glu Leu Pro Glu
 565 570 575
 Phe Leu Leu Phe Leu Gln His Ala Ile Ser Val Pro Glu Asp Val Ala
 580 585 590
 Arg Asp Leu Gly Asp Val Met Glu Thr Val Leu Ser Ser Gln Thr Cys
 595 600 605
 Glu Gln Thr Pro Glu Arg Leu Phe Val Pro Ser Cys Thr Thr Glu Gly
 610 615 620
 Ser Tyr Glu Asp Val Gln Cys Phe Ser Gly Glu Cys Trp Cys Val Asn
 625 630 635 640
 Ser Trp Gly Lys Glu Leu Pro Gly Ser Arg Val Arg Gly Gly Gln Pro
 645 650 655
 Arg Cys Pro Thr Asp Cys Glu Lys Gln Arg Ala Arg Met Gln Ser Leu
 660 665 670
 Met Gly Ser Gln Pro Ala Gly Ser Thr Leu Phe Val Pro Ala Cys Thr
 675 680 685
 Ser Glu Gly His Phe Leu Pro Val Gln Cys Phe Asn Ser Glu Cys Tyr
 690 695 700
 Cys Val Asp Ala Glu Gly Gln Ala Ile Pro Gly Thr Arg Ser Ala Ile
 705 710 715 720
 Gly Lys Pro Lys Lys Cys Pro Thr Pro Cys Gln Leu Gln Ser Glu Gln
 725 730 735
 Ala Phe Leu Arg Thr Val Gln Ala Leu Leu Ser Asn Ser Ser Met Leu
 740 745 750
 Pro Thr Leu Ser Asp Thr Tyr Ile Pro Gln Cys Ser Thr Asp Gly Gln
 755 760 765
 Trp Arg Gln Val Gln Cys Asn Gly Pro Pro Glu Gln Val Phe Glu Leu
 770 775 780
 Tyr Gln Arg Trp Glu Ala Gln Asn Lys Gly Gln Asp Leu Thr Pro Ala
 785 790 795 800
 Lys Leu Leu Val Lys Ile Met Ser Tyr Arg Glu Ala Ala Ser Gly Asn
 805 810 815
 Phe Ser Leu Phe Ile Gln Ser Leu Tyr Glu Ala Gly Gln Gln Asp Val
 820 825 830
 Phe Pro Val Leu Ser Gln Tyr Pro Ser Leu Gln Asp Val Pro Leu Ala

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835					840					845					
Ala	Leu	Glu	Gly	Lys	Arg	Pro	Gln	Pro	Arg	Glu	Asn	Ile	Leu	Leu	Glu
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Pro	Tyr	Leu	Phe	Trp	Gln	Ile	Leu	Asn	Gly	Gln	Leu	Ser	Gln	Tyr	Pro
865					870				875						880
Gly	Ser	Tyr	Ser	Asp	Phe	Ser	Thr	Pro	Leu	Ala	His	Phe	Asp	Leu	Arg
				885					890					895	
Asn	Cys	Trp	Cys	Val	Asp	Glu	Ala	Gly	Gln	Glu	Leu	Glu	Gly	Met	Arg
			900					905					910		
Ser	Glu	Pro	Ser	Lys	Leu	Pro	Thr	Cys	Pro	Gly	Ser	Cys	Glu	Glu	Ala
		915					920					925			
Lys	Leu	Arg	Val	Leu	Gln	Phe	Ile	Arg	Glu	Thr	Glu	Glu	Ile	Val	Ser
930						935					940				
Ala	Ser	Asn	Ser	Ser	Arg	Phe	Pro	Leu	Gly	Glu	Ser	Phe	Leu	Val	Ala
945					950					955					960
Lys	Gly	Ile	Arg	Leu	Arg	Asn	Glu	Asp	Leu	Gly	Leu	Pro	Pro	Leu	Phe
				965					970					975	
Pro	Pro	Arg	Glu	Ala	Phe	Ala	Glu	Gln	Phe	Leu	Arg	Gly	Ser	Asp	Tyr
			980					985						990	
Ala	Ile	Arg	Leu	Ala	Ala	Gln	Ser	Thr	Leu	Ser	Phe	Tyr	Gln	Arg	Arg
		995					1000						1005		
Arg	Phe	Ser	Pro	Asp	Asp	Ser	Ala	Gly	Ala	Ser	Ala	Leu	Leu	Arg	
1010						1015					1020				
Ser	Gly	Pro	Tyr	Met	Pro	Gln	Cys	Asp	Ala	Phe	Gly	Ser	Trp	Glu	
1025						1030					1035				
Pro	Val	Gln	Cys	His	Ala	Gly	Thr	Gly	His	Cys	Trp	Cys	Val	Asp	
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Glu	Lys	Gly	Gly	Phe	Ile	Pro	Gly	Ser	Leu	Thr	Ala	Arg	Ser	Leu	
1055						1060					1065				
Gln	Ile	Pro	Gln	Cys	Pro	Thr	Thr	Cys	Glu	Lys	Ser	Arg	Thr	Ser	
1070						1075					1080				
Gly	Leu	Leu	Ser	Ser	Trp	Lys	Gln	Ala	Arg	Ser	Gln	Glu	Asn	Pro	
1085						1090					1095				
Ser	Pro	Lys	Asp	Leu	Phe	Val	Pro	Ala	Cys	Leu	Glu	Thr	Gly	Glu	
1100						1105					1110				
Tyr	Ala	Arg	Leu	Gln	Ala	Ser	Gly	Ala	Gly	Thr	Trp	Cys	Val	Asp	
1115						1120					1125				
Pro	Ala	Ser	Gly	Glu	Glu	Leu	Arg	Pro	Gly	Ser	Ser	Ser	Ser	Ala	
1130						1135					1140				
Gln	Cys	Pro	Ser	Leu	Cys	Asn	Val	Leu	Lys	Ser	Gly	Val	Leu	Ser	
1145						1150					1155				
Arg	Arg	Val	Ser	Pro	Gly	Tyr	Val	Pro	Ala	Cys	Arg	Ala	Glu	Asp	
1160						1165					1170				
Gly	Gly	Phe	Ser	Pro	Val	Gln	Cys	Asp	Gln	Ala	Gln	Gly	Ser	Cys	
1175						1180					1185				
Trp	Cys	Val	Met	Asp	Ser	Gly	Glu	Glu	Val	Pro	Gly	Thr	Arg	Val	
1190						1195					1200				
Thr	Gly	Gly	Gln	Pro	Ala	Cys	Glu	Ser	Pro	Arg	Cys	Pro	Leu	Pro	
1205						1210					1215				
Phe	Asn	Ala	Ser	Glu	Val	Val	Gly	Gly	Thr	Ile	Leu	Cys	Glu	Thr	
1220						1225					1230				
Ile	Ser	Gly	Pro	Thr	Gly	Ser	Ala	Met	Gln	Gln	Cys	Gln	Leu	Leu	
1235						1240					1245				

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Cys Arg	Gln Gly	Ser Trp	Ser Val	Phe Pro	Pro Gly	Pro Leu	Ile						
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Cys Ser	Leu Glu	Ser Gly	Arg Trp	Glu Ser	Gln Leu	Pro Gln	Pro						
1265			1270				1275						
Arg Ala	Cys Gln	Arg Pro	Gln Leu	Trp Gln	Thr Ile	Gln Thr	Gln						
1280			1285				1290						
Gly His	Phe Gln	Leu Gln	Leu Pro	Pro Gly	Lys Met	Cys Ser	Ala						
1295			1300				1305						
Asp Tyr	Ala Asp	Leu Leu	Gln Thr	Phe Gln	Val Phe	Ile Leu	Asp						
1310			1315				1320						
Glu Leu	Thr Ala	Arg Gly	Phe Cys	Gln Ile	Gln Val	Lys Thr	Phe						
1325			1330				1335						
Gly Thr	Leu Val	Ser Ile	Pro Val	Cys Asn	Asn Ser	Ser Val	Gln						
1340			1345				1350						
Val Gly	Cys Leu	Thr Arg	Glu Arg	Leu Gly	Val Asn	Val Thr	Trp						
1355			1360				1365						
Lys Ser	Arg Leu	Glu Asp	Ile Pro	Val Ala	Ser Leu	Pro Asp	Leu						
1370			1375				1380						
His Asp	Ile Glu	Arg Ala	Leu Val	Gly Lys	Asp Leu	Leu Gly	Arg						
1385			1390				1395						
Phe Thr	Asp Leu	Ile Gln	Ser Gly	Ser Phe	Gln Leu	His Leu	Asp						
1400			1405				1410						
Ser Lys	Thr Phe	Pro Ala	Glu Thr	Ile Arg	Phe Leu	Gln Gly	Asp						
1415			1420				1425						
His Phe	Gly Thr	Ser Pro	Arg Thr	Trp Phe	Gly Cys	Ser Glu	Gly						
1430			1435				1440						
Phe Tyr	Gln Val	Leu Thr	Ser Glu	Ala Ser	Gln Asp	Gly Leu	Gly						
1445			1450				1455						
Cys Val	Lys Cys	Pro Glu	Gly Ser	Tyr Ser	Gln Asp	Glu Glu	Cys						
1460			1465				1470						
Ile Pro	Cys Pro	Val Gly	Phe Tyr	Gln Glu	Gln Ala	Gly Ser	Leu						
1475			1480				1485						
Ala Cys	Val Pro	Cys Pro	Val Gly	Arg Thr	Thr Ile	Ser Ala	Gly						
1490			1495				1500						
Ala Phe	Ser Gln	Thr His	Leu Met	Gln Lys	Phe Glu	Lys Val	Pro						
1505			1510				1515						
Glu Ser	Lys Val	Ile Phe	Asp Ala	Asn Ala	Pro Val	Ala Val	Arg						
1520			1525				1530						
Ser Lys	Val Pro	Asp Ser	Glu Phe	Pro Val	Met Gln	Cys Leu	Thr						
1535			1540				1545						
Asp Cys	Thr Glu	Asp Glu	Ala Cys	Ser Phe	Phe Thr	Val Ser	Thr						
1550			1555				1560						
Thr Glu	Pro Glu	Ile Ser	Cys Asp	Phe Tyr	Ala Trp	Thr Ser	Asp						
1565			1570				1575						
Asn Val	Ala Cys	Met Thr	Ser Asp	Gln Lys	Arg Asp	Ala Leu	Gly						
1580			1585				1590						
Asn Ser	Lys Ala	Thr Ser	Phe Gly	Ser Leu	Arg Cys	Gln Val	Lys						
1595			1600				1605						
Val Arg	Ser His	Gly Gln	Asp Ser	Pro Ala	Val Tyr	Leu Lys	Lys						
1610			1615				1620						
Gly Gln	Gly Ser	Thr Thr	Thr Leu	Gln Lys	Arg Phe	Glu Pro	Thr						
1625			1630				1635						
Gly Phe	Gln Asn	Met Leu	Ser Gly	Leu Tyr	Asn Pro	Ile Val	Phe						
1640			1645				1650						

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Ser Ala	Ser Gly	Ala Asn	Leu Thr	Asp Ala	His Leu	Phe Cys	Leu						
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Leu Ala	Cys Asp	Arg Asp	Leu Cys	Cys Asp	Gly Phe	Val Leu	Thr						
1670			1675			1680							
Gln Val	Gln Gly	Gly Ala	Ile Ile	Cys Gly	Leu Leu	Ser Ser	Pro						
1685			1690			1695							
Ser Val	Leu Leu	Cys Asn	Val Lys	Asp Trp	Met Asp	Pro Ser	Glu						
1700			1705			1710							
Ala Trp	Ala Asn	Ala Thr	Cys Pro	Gly Val	Thr Tyr	Asp Gln	Glu						
1715			1720			1725							
Ser His	Gln Val	Ile Leu	Arg Leu	Gly Asp	Gln Glu	Phe Ile	Lys						
1730			1735			1740							
Ser Leu	Thr Pro	Leu Glu	Gly Thr	Gln Asp	Thr Phe	Thr Asn	Phe						
1745			1750			1755							
Gln Gln	Val Tyr	Leu Trp	Lys Asp	Ser Asp	Met Gly	Ser Arg	Pro						
1760			1765			1770							
Glu Ser	Met Gly	Cys Arg	Lys Asp	Thr Val	Pro Arg	Pro Ala	Ser						
1775			1780			1785							
Pro Thr	Glu Ala	Gly Leu	Thr Thr	Glu Leu	Phe Ser	Pro Val	Asp						
1790			1795			1800							
Leu Asn	Gln Val	Ile Val	Asn Gly	Asn Gln	Ser Leu	Ser Ser	Gln						
1805			1810			1815							
Lys His	Trp Leu	Phe Lys	His Leu	Phe Ser	Ala Gln	Gln Ala	Asn						
1820			1825			1830							
Leu Trp	Cys Leu	Ser Arg	Cys Val	Gln Glu	His Ser	Phe Cys	Gln						
1835			1840			1845							
Leu Ala	Glu Ile	Thr Glu	Ser Ala	Ser Leu	Tyr Phe	Thr Cys	Thr						
1850			1855			1860							
Leu Tyr	Pro Glu	Ala Gln	Val Cys	Asp Asp	Ile Met	Glu Ser	Asn						
1865			1870			1875							
Ala Gln	Gly Cys	Arg Leu	Ile Leu	Pro Gln	Met Pro	Lys Ala	Leu						
1880			1885			1890							
Phe Arg	Lys Lys	Val Ile	Leu Glu	Asp Lys	Val Lys	Asn Phe	Tyr						
1895			1900			1905							
Thr Arg	Leu Pro	Phe Gln	Lys Leu	Met Gly	Ile Ser	Ile Arg	Asn						
1910			1915			1920							
Lys Val	Pro Met	Ser Glu	Lys Ser	Ile Ser	Asn Gly	Phe Phe	Glu						
1925			1930			1935							
Cys Glu	Arg Arg	Cys Asp	Ala Asp	Pro Cys	Cys Thr	Gly Phe	Gly						
1940			1945			1950							
Phe Leu	Asn Val	Ser Gln	Leu Lys	Gly Gly	Glu Val	Thr Cys	Leu						
1955			1960			1965							
Thr Leu	Asn Ser	Leu Gly	Ile Gln	Met Cys	Ser Glu	Glu Asn	Gly						
1970			1975			1980							
Gly Ala	Trp Arg	Ile Leu	Asp Cys	Gly Ser	Pro Asp	Ile Glu	Val						
1985			1990			1995							
His Thr	Tyr Pro	Phe Gly	Trp Tyr	Gln Lys	Pro Ile	Ala Gln	Asn						
2000			2005			2010							
Asn Ala	Pro Ser	Phe Cys	Pro Leu	Val Val	Leu Pro	Ser Leu	Thr						
2015			2020			2025							
Glu Lys	Val Ser	Leu Asp	Ser Trp	Gln Ser	Leu Ala	Leu Ser	Ser						
2030			2035			2040							
Val Val	Val Asp	Pro Ser	Ile Arg	His Phe	Asp Val	Ala His	Val						

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Leu Leu Ile Gly Ser Ser Gln Asp Asp Gly Leu Ile Asn Arg Ala
 2450 2455 2460
 Lys Ala Val Lys Gln Phe Glu Glu Ser Arg Gly Arg Thr Ser Ser
 2465 2470 2475
 Lys Thr Ala Phe Tyr Gln Ala Leu Gln Asn Ser Leu Gly Gly Glu
 2480 2485 2490
 Asp Ser Asp Ala Arg Val Glu Ala Ala Ala Thr Trp Tyr Tyr Ser
 2495 2500 2505
 Leu Glu His Ser Thr Asp Asp Tyr Ala Ser Phe Ser Arg Ala Leu
 2510 2515 2520
 Glu Asn Ala Thr Arg Asp Tyr Phe Ile Ile Cys Pro Ile Ile Asp
 2525 2530 2535
 Met Ala Ser Ala Trp Ala Lys Arg Ala Arg Gly Asn Val Phe Met
 2540 2545 2550
 Tyr His Ala Pro Glu Asn Tyr Gly His Gly Ser Leu Glu Leu Leu
 2555 2560 2565
 Ala Asp Val Gln Phe Ala Leu Gly Leu Pro Phe Tyr Pro Ala Tyr
 2570 2575 2580
 Glu Gly Gln Phe Ser Leu Glu Glu Lys Ser Leu Ser Leu Lys Ile
 2585 2590 2595
 Met Gln Tyr Phe Ser His Phe Ile Arg Ser Gly Asn Pro Asn Tyr
 2600 2605 2610
 Pro Tyr Glu Phe Ser Arg Lys Val Pro Thr Phe Ala Thr Pro Trp
 2615 2620 2625
 Pro Asp Phe Val Pro Arg Ala Gly Gly Glu Asn Tyr Lys Glu Phe
 2630 2635 2640
 Ser Glu Leu Leu Pro Asn Arg Gln Gly Leu Lys Lys Ala Asp Cys
 2645 2650 2655
 Ser Phe Trp Ser Lys Tyr Ile Ser Ser Leu Lys Thr Ser Ala Asp
 2660 2665 2670
 Gly Ala Lys Gly Gly Gln Ser Ala Glu Ser Glu Glu Glu Glu Leu
 2675 2680 2685
 Thr Ala Gly Ser Gly Leu Arg Glu Asp Leu Leu Ser Leu Gln Glu
 2690 2695 2700
 Pro Gly Ser Lys Thr Tyr Ser Lys
 2705 2710

<210> SEQ ID NO 3
 <211> LENGTH: 1574
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Ile Pro Arg Lys Pro Ile Ser Lys Arg Pro Val Arg Pro Ser Leu Pro
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 Arg Ser Pro Arg Cys Pro Leu Pro Phe Asn Ala Ser Glu Val Val Gly
 20 25 30
 Gly Thr Ile Leu Cys Glu Thr Ile Ser Gly Pro Thr Gly Ser Ala Met
 35 40 45
 Gln Gln Cys Gln Leu Leu Cys Arg Gln Gly Ser Trp Ser Val Phe Pro
 50 55 60
 Pro Gly Pro Leu Ile Cys Ser Leu Glu Ser Gly Arg Trp Glu Ser Gln
 65 70 75 80
 Leu Pro Gln Pro Arg Ala Cys Gln Arg Pro Gln Leu Trp Gln Thr Ile
 85 90 95

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Gln	Thr	Gln	Gly	His	Phe	Gln	Leu	Gln	Leu	Pro	Pro	Gly	Lys	Met	Cys
			100					105					110		
Ser	Ala	Asp	Tyr	Ala	Gly	Leu	Leu	Gln	Thr	Phe	Gln	Val	Phe	Ile	Leu
		115					120					125			
Asp	Glu	Leu	Thr	Ala	Arg	Gly	Phe	Cys	Gln	Ile	Gln	Val	Lys	Thr	Phe
	130					135					140				
Gly	Thr	Leu	Val	Ser	Ile	Pro	Val	Cys	Asn	Asn	Ser	Ser	Val	Gln	Val
145					150					155					160
Gly	Cys	Leu	Thr	Arg	Glu	Arg	Leu	Gly	Val	Asn	Val	Thr	Trp	Lys	Ser
				165					170					175	
Arg	Leu	Glu	Asp	Ile	Pro	Val	Ala	Ser	Leu	Pro	Asp	Leu	His	Asp	Ile
			180					185					190		
Glu	Arg	Ala	Leu	Val	Gly	Lys	Asp	Leu	Leu	Gly	Arg	Phe	Thr	Asp	Leu
		195					200					205			
Ile	Gln	Ser	Gly	Ser	Phe	Gln	Leu	His	Leu	Asp	Ser	Lys	Thr	Phe	Pro
	210					215					220				
Ala	Glu	Thr	Ile	Arg	Phe	Leu	Gln	Gly	Asp	His	Phe	Gly	Thr	Ser	Pro
225					230					235					240
Arg	Thr	Trp	Phe	Gly	Cys	Ser	Glu	Gly	Phe	Tyr	Gln	Val	Leu	Thr	Ser
				245					250					255	
Glu	Ala	Ser	Gln	Asp	Gly	Leu	Gly	Cys	Val	Lys	Cys	Pro	Glu	Gly	Ser
			260					265					270		
Tyr	Ser	Gln	Asp	Glu	Glu	Cys	Ile	Pro	Cys	Pro	Val	Gly	Phe	Tyr	Gln
		275					280					285			
Glu	Gln	Ala	Gly	Ser	Leu	Ala	Cys	Val	Pro	Cys	Pro	Val	Gly	Arg	Thr
	290					295					300				
Thr	Ile	Ser	Ala	Gly	Ala	Phe	Ser	Gln	Thr	His	Cys	Val	Thr	Asp	Cys
305					310					315					320
Gln	Arg	Asn	Glu	Ala	Gly	Leu	Gln	Cys	Asp	Gln	Asn	Gly	Gln	Tyr	Arg
				325					330					335	
Ala	Ser	Gln	Lys	Asp	Arg	Gly	Ser	Gly	Lys	Ala	Phe	Cys	Val	Asp	Gly
			340					345					350		
Glu	Gly	Arg	Arg	Leu	Pro	Trp	Trp	Glu	Thr	Glu	Ala	Pro	Leu	Glu	Asp
		355					360					365			
Ser	Gln	Cys	Leu	Met	Met	Gln	Lys	Phe	Glu	Lys	Val	Pro	Glu	Ser	Lys
	370					375					380				
Val	Ile	Phe	Asp	Ala	Asn	Ala	Pro	Val	Ala	Val	Arg	Ser	Lys	Val	Pro
385					390					395					400
Asp	Ser	Glu	Phe	Pro	Val	Met	Gln	Cys	Leu	Thr	Asp	Cys	Thr	Glu	Asp
				405					410					415	
Glu	Ala	Cys	Ser	Phe	Phe	Thr	Val	Ser	Thr	Thr	Glu	Pro	Glu	Ile	Ser
				420				425					430		
Cys	Asp	Phe	Tyr	Ala	Trp	Thr	Ser	Asp	Asn	Val	Ala	Cys	Met	Thr	Ser
		435					440					445			
Asp	Gln	Lys	Arg	Asp	Ala	Leu	Gly	Asn	Ser	Lys	Ala	Thr	Ser	Phe	Gly
	450					455					460				
Ser	Leu	Arg	Cys	Gln	Val	Lys	Val	Arg	Ser	His	Gly	Gln	Asp	Ser	Pro
465					470					475					480
Ala	Val	Tyr	Leu	Lys	Lys	Gly	Gln	Gly	Ser	Thr	Thr	Thr	Leu	Gln	Lys
				485					490					495	
Arg	Phe	Glu	Pro	Thr	Gly	Phe	Gln	Asn	Met	Leu	Ser	Gly	Leu	Tyr	Asn
			500					505					510		
Pro	Ile	Val	Phe	Ser	Ala	Ser	Gly	Ala	Asn	Leu	Thr	Asp	Ala	His	Leu
		515					520						525		

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Phe Cys Leu Leu Ala Cys Asp Arg Asp Leu Cys Cys Asp Gly Phe Val
530 535 540
Leu Thr Gln Val Gln Gly Gly Ala Ile Ile Cys Gly Leu Leu Ser Ser
545 550 555 560
Pro Ser Val Leu Leu Cys Asn Val Lys Asp Trp Met Asp Pro Ser Glu
565 570 575
Ala Trp Ala Asn Ala Thr Cys Pro Gly Val Thr Tyr Asp Gln Glu Ser
580 585 590
His Gln Val Ile Leu Arg Leu Gly Asp Gln Glu Phe Ile Lys Ser Leu
595 600 605
Thr Pro Leu Glu Gly Thr Gln Asp Thr Phe Thr Asn Phe Gln Gln Val
610 615 620
Tyr Leu Trp Lys Asp Ser Asp Met Gly Ser Arg Pro Glu Ser Met Gly
625 630 635 640
Cys Arg Lys Asn Thr Val Pro Arg Pro Ala Ser Pro Thr Glu Ala Gly
645 650 655
Leu Thr Thr Glu Leu Phe Ser Pro Val Asp Leu Asn Gln Val Ile Val
660 665 670
Asn Gly Asn Gln Ser Leu Ser Ser Gln Lys His Trp Leu Phe Lys His
675 680 685
Leu Phe Ser Ala Gln Gln Ala Asn Leu Trp Cys Leu Ser Arg Cys Val
690 695 700
Gln Glu His Ser Phe Cys Gln Leu Ala Glu Ile Thr Glu Ser Ala Ser
705 710 715 720
Leu Tyr Phe Thr Cys Thr Leu Tyr Pro Glu Ala Gln Val Cys Asp Asp
725 730 735
Ile Met Glu Ser Asn Ala Gln Gly Cys Arg Leu Ile Leu Pro Gln Met
740 745 750
Pro Lys Ala Leu Phe Arg Lys Lys Val Ile Leu Glu Asp Lys Val Lys
755 760 765
Asn Phe Tyr Thr Arg Leu Pro Phe Gln Lys Leu Thr Gly Ile Ser Ile
770 775 780
Arg Asn Lys Val Pro Met Ser Glu Lys Ser Ile Ser Asn Gly Phe Phe
785 790 795 800
Glu Cys Glu Arg Arg Cys Asp Ala Asp Pro Cys Cys Thr Gly Phe Gly
805 810 815
Phe Leu Asn Val Ser Gln Leu Lys Gly Gly Glu Val Thr Cys Leu Thr
820 825 830
Leu Asn Ser Leu Gly Ile Gln Met Cys Ser Glu Glu Asn Gly Gly Ala
835 840 845
Trp Arg Ile Leu Asp Cys Gly Ser Pro Asp Ile Glu Val His Thr Tyr
850 855 860
Pro Phe Gly Trp Tyr Gln Lys Pro Ile Ala Gln Asn Asn Ala Pro Ser
865 870 875 880
Phe Cys Pro Leu Val Val Leu Pro Ser Leu Thr Glu Lys Val Ser Leu
885 890 895
Asp Ser Trp Gln Ser Leu Ala Leu Ser Ser Val Val Val Asp Pro Ser
900 905 910
Ile Arg His Phe Asp Val Ala His Val Ser Thr Ala Ala Thr Ser Asn
915 920 925
Phe Ser Ala Val Arg Asp Leu Cys Leu Ser Glu Cys Ser Gln His Glu
930 935 940
Ala Cys Leu Ile Thr Thr Leu Gln Thr Gln Pro Gly Ala Val Arg Cys

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945	950	955	960
Met Phe Tyr Ala Asp Thr Gln Ser Cys Thr His Ser Leu Gln Gly Gln	965	970	975
Asn Cys Arg Leu Leu Arg Glu Glu Ala Thr His Ile Tyr Arg Lys	980	985	990
Pro Gly Ile Ser Leu Leu Ser Tyr Glu Ala Ser Val Pro Ser Val Pro	995	1000	1005
Ile Ser Thr His Gly Arg Leu Leu Gly Arg Ser Gln Ala Ile Gln	1010	1015	1020
Val Gly Thr Ser Trp Lys Gln Val Asp Gln Phe Leu Gly Val Pro	1025	1030	1035
Tyr Ala Ala Pro Pro Leu Ala Glu Arg Arg Phe Gln Ala Pro Glu	1040	1045	1050
Pro Leu Asn Trp Thr Gly Ser Trp Asp Ala Ser Lys Pro Arg Ala	1055	1060	1065
Ser Cys Trp Gln Pro Gly Thr Arg Thr Ser Thr Ser Pro Gly Val	1070	1075	1080
Ser Glu Asp Cys Leu Tyr Leu Asn Val Phe Ile Pro Gln Asn Val	1085	1090	1095
Ala Pro Asn Ala Ser Val Leu Val Phe Phe His Asn Thr Met Asp	1100	1105	1110
Arg Glu Glu Ser Glu Gly Trp Pro Ala Ile Asp Gly Ser Phe Leu	1115	1120	1125
Ala Ala Val Gly Asn Leu Ile Val Val Thr Ala Ser Tyr Arg Val	1130	1135	1140
Gly Val Phe Gly Phe Leu Ser Ser Gly Ser Gly Glu Val Ser Gly	1145	1150	1155
Asn Trp Gly Leu Leu Asp Gln Val Ala Ala Leu Thr Trp Val Gln	1160	1165	1170
Thr His Ile Arg Gly Phe Gly Gly Asp Pro Arg Arg Val Ser Leu	1175	1180	1185
Ala Ala Asp Arg Gly Gly Ala Asp Val Ala Ser Ile His Leu Leu	1190	1195	1200
Thr Ala Arg Ala Thr Asn Ser Gln Leu Phe Arg Arg Ala Val Leu	1205	1210	1215
Met Gly Gly Ser Ala Leu Ser Pro Ala Ala Val Ile Ser His Glu	1220	1225	1230
Arg Ala Gln Gln Gln Ala Ile Ala Leu Ala Lys Glu Val Ser Cys	1235	1240	1245
Pro Met Ser Ser Ser Gln Glu Val Val Ser Cys Leu Arg Gln Lys	1250	1255	1260
Pro Ala Asn Val Leu Asn Asp Ala Gln Thr Lys Leu Leu Ala Val	1265	1270	1275
Ser Gly Pro Phe His Tyr Trp Gly Pro Val Ile Asp Gly His Phe	1280	1285	1290
Leu Arg Glu Pro Pro Ala Arg Ala Leu Lys Arg Ser Leu Trp Val	1295	1300	1305
Glu Val Asp Leu Leu Ile Gly Ser Ser Gln Asp Asp Gly Leu Ile	1310	1315	1320
Asn Arg Ala Lys Ala Val Lys Gln Phe Glu Glu Ser Gln Gly Arg	1325	1330	1335
Thr Ser Ser Lys Thr Ala Phe Tyr Gln Ala Leu Gln Asn Ser Leu	1340	1345	1350

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Gly	Gly	Glu	Asp	Ser	Asp	Ala	Arg	Val	Glu	Ala	Ala	Ala	Thr	Trp
	1355					1360					1365			
Tyr	Tyr	Ser	Leu	Glu	His	Ser	Thr	Asp	Asp	Tyr	Ala	Ser	Phe	Ser
	1370					1375					1380			
Arg	Ala	Leu	Glu	Asn	Ala	Thr	Arg	Asp	Tyr	Phe	Ile	Ile	Cys	Pro
	1385					1390					1395			
Ile	Ile	Asp	Met	Ala	Ser	Ala	Trp	Ala	Lys	Arg	Ala	Arg	Gly	Asn
	1400					1405					1410			
Val	Phe	Met	Tyr	His	Ala	Pro	Glu	Asn	Tyr	Gly	His	Gly	Ser	Leu
	1415					1420					1425			
Glu	Leu	Leu	Ala	Asp	Val	Gln	Phe	Ala	Leu	Gly	Leu	Pro	Phe	Tyr
	1430					1435					1440			
Pro	Ala	Tyr	Glu	Gly	Gln	Phe	Ser	Leu	Glu	Glu	Lys	Ser	Leu	Ser
	1445					1450					1455			
Leu	Lys	Ile	Met	Gln	Tyr	Phe	Ser	His	Phe	Ile	Arg	Ser	Gly	Asn
	1460					1465					1470			
Pro	Asn	Tyr	Pro	Tyr	Glu	Phe	Ser	Arg	Lys	Val	Pro	Thr	Phe	Ala
	1475					1480					1485			
Thr	Pro	Trp	Pro	Asp	Phe	Val	Pro	Arg	Ala	Gly	Gly	Glu	Asn	Tyr
	1490					1495					1500			
Lys	Glu	Phe	Ser	Glu	Leu	Leu	Pro	Asn	Arg	Gln	Gly	Leu	Lys	Lys
	1505					1510					1515			
Ala	Asp	Cys	Ser	Phe	Trp	Ser	Lys	Tyr	Ile	Ser	Ser	Leu	Lys	Thr
	1520					1525					1530			
Ser	Ala	Asp	Gly	Ala	Lys	Gly	Gly	Gln	Ser	Ala	Glu	Ser	Glu	Glu
	1535					1540					1545			
Glu	Glu	Leu	Thr	Ala	Gly	Ser	Gly	Leu	Arg	Glu	Asp	Leu	Leu	Ser
	1550					1555					1560			
Leu	Gln	Glu	Pro	Gly	Ser	Lys	Thr	Tyr	Ser	Lys				
	1565					1570								

<210> SEQ ID NO 4
 <211> LENGTH: 12
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Val	Ile	Phe	Asp	Ala	Asn	Ala	Pro	Val	Ala	Val	Arg
1				5					10		

That which is claimed is:

1. A method for determining the amount of thyroglobulin ⁵⁰ in a test sample, comprising:

- (a) digesting thyroglobulin from said test sample to form peptide T129;
- (b) purifying said peptide T129 from step (a);
- (c) ionizing said peptide T129 from step (b) to generate a ⁵⁵ multiply charged ion detectable by mass spectrometry;
- (d) detecting the amount of one or more ions by mass spectrometry; wherein the amount of said ion(s) detected is related to the amount of said thyroglobulin in ⁶⁰ said test sample.

2. The method of claim 1, wherein said ion of step (c) has a mass/charge ratio of 636.4±0.5.

3. The method of claim 1, wherein one or more of said ions ⁶⁵ detected in step (d) are selected from the group consisting of ions with m/z of 541.3±0.5, 612.3±0.5, 636.4±0.5, 726.4±0.5, 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

4. The method of claim 1, wherein one or more of said ions ⁵⁰ detected in step (d) are selected from the group consisting of ions with m/z of 636.4±0.5, 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

5. The method of claim 1, wherein said mass spectrometry comprises tandem mass spectrometry.

6. The method of claim 5, wherein said ionizing of step (c) ⁵⁵ comprises:

- ionizing said peptide T129 from step (b) to generate a multiply charged peptide T129 precursor ion detectable by mass spectrometry;
- and colliding said peptide T129 precursor ion with a collision gas to generate one or more peptide T129 fragment ions.

7. The method of claim 6, wherein said one or more peptide ⁶⁵ T129 fragment ions are selected from the group consisting of ions with m/z of 541.3±0.5, 612.3±0.5, 726.4±0.5, 797.4±0.5, 912.4±0.5, and 1059.5±0.5.

8. The method of claim 6, wherein said one or more peptide T129 fragment ions are selected from the group consisting of ions with m/z of 797.4 ± 0.5 , 912.4 ± 0.5 , and 1059.5 ± 0.5 .

9. The method of claim 6, wherein said ions detected in step (d) comprise one or more peptide T129 fragment ions.

10. The method of claim 1, wherein step (b) is accomplished by utilizing at least one size separation technique.

11. The method of claim 1, further comprising purifying thyroglobulin from a body fluid or tissue sample to generate a thyroglobulin containing test sample.

12. The method of claim 11, wherein said body fluid or tissue sample comprises plasma or serum.

13. The method of claim 1, wherein said test sample comprises a body fluid.

14. The method of claim 13, wherein said test sample comprises plasma or serum.

15. A method for determining persistence of thyroid tissue in a patient after surgical removal of the thyroid gland, the method comprising:

(a) providing a body fluid or tissue sample obtained from a patient;

(b) processing said sample to generate a processed sample, wherein at least one step in said processing comprises subjecting said processed sample to trypsin under conditions suitable to digest thyroglobulin and thereby generate peptide T129, and at least a second step in said processing comprises subjecting the post-digested processed sample to one or more purification steps under conditions suitable to enrich peptide T129;

(c) subjecting the processed sample of step (b) to an ionization source under conditions suitable to generate a multiply charged peptide T129 ion detectable by mass spectrometry;

(d) detecting the presence of one or more peptide T129 ions by mass spectrometry;

wherein the presence of said one or more peptide T129 ions indicates persistence of thyroid tissue in the patient.

16. The method of claim 15, wherein said ion of step (c) has a mass/charge ratio of 636.4 ± 0.5 .

17. The method of claim 15, wherein one or more of said ions detected in step (d) are selected from the group consisting of ions with m/z of 541.3 ± 0.5 , 612.3 ± 0.5 , 636.4 ± 0.5 , 726.4 ± 0.5 , 797.4 ± 0.5 , 912.4 ± 0.5 , and 1059.5 ± 0.5 .

18. The method of claim 15, wherein one or more of said ions detected in step (d) are selected from the group consisting of ions with m/z of 636.4 ± 0.5 , 797.4 ± 0.5 , 912.4 ± 0.5 , and 1059.5 ± 0.5 .

19. The method of claim 15, wherein said mass spectrometry comprises tandem mass spectrometry.

20. The method of claim 19, wherein said ionizing of step (c) comprises:

ionizing said peptide T129 from step (b) to generate a multiply charged peptide T129 precursor ion detectable by mass spectrometry;

and colliding said peptide T129 precursor ion with a collision gas to generate one or more peptide T129 fragment ions.

21. The method of claim 20, wherein said one or more peptide T129 fragment ions are selected from the group consisting of ions with m/z of 541.3 ± 0.5 , 612.3 ± 0.5 , 726.4 ± 0.5 , 797.4 ± 0.5 , 912.4 ± 0.5 , and 1059.5 ± 0.5 .

22. The method of claim 20, wherein said one or more peptide T129 fragment ions are selected from the group consisting of ions with m/z of 797.4 ± 0.5 , 912.4 ± 0.5 , and 1059.5 ± 0.5 .

23. The method of claim 20, wherein said ions detected in step (d) comprise one or more peptide T129 fragment ions.

24. The method of claim 15, wherein step (b) is accomplished by utilizing at least one size separation technique.

25. The method of claim 15, further comprising purifying thyroglobulin from a body fluid or tissue sample to generate a thyroglobulin containing test sample.

26. The method of claim 25, wherein said body fluid or tissue sample comprises plasma or serum.

27. The method of claim 15, wherein said test sample comprises a body fluid.

28. The method of claim 27, wherein said test sample comprises plasma or serum.

* * * * *